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CLINICAL AND EXPERIMENTAL

THE VALUE OF AQUEOUS EQUINE LIVER EXTRACT, GLYCERATED IRON, AND HEMOGLOBIN IN THE TREATMENT OF SECONDARY ANEMIAS*

OSCAR RICHTER, M.D., ARTHUR E. MEYER, PH.D., AND MISS HELEN LEGERE, CHICAGO

WE HAVE recently reported[1, 2] on the presence of the "antipernicious anemia" principle in the liver of the horse. Both the oral and subcutaneous equine liver extracts containing the active principle soluble in 70 per cent alcohol, described by Cohn[3] in the preparation of fraction G., produced prompt hematologic remissions when administered to pernicious anemia patients. The benefit of this fraction in the treatment of anemias of the pernicious type, including sprue, fish-tapeworm infestations, certain cryptogenic hyperchromatic anemias, and chronic anemias of long standing dietary deficiency diseases, has been established. Giffin's[4] suggestion that liver extract may be beneficial in the treatment of anemias associated with organic gastric disease was not corroborated by Castle's[5-7] recent work. These investigators were able to demonstrate that the activating substance absent in the gastric secretion of patients with pernicious anemia, was present in four patients with anemia having a total anacidity, and absent in two patients with pernicious anemia-like blood pictures having an apparently normal gastric juice; the former responded to iron therapy; the latter responded to liver extract therapy.

It is generally recognized that the 70 per cent alcohol-soluble fraction of liver is not effective or only mildly effective in the treatment of secondary anemia, in contrast to the frequent good responses obtained from the feeding of whole liver. Robscheit-Robbins[8] demonstrated in experimental posthemorrhagic anemias that the alcohol-soluble liver extract only retained 15 per cent of the potency of whole

*This work was made possible by a grant from the Chappel Foundation for Organotherapeutic Research and was done at Northwestern University and Cook County Hospital.

liver. Furth and Singer[9] were unable to obtain any effect on experimentally induced anemias with Minot's liver extract, and found the regeneration period definitely shortened when whole liver was added. Earlier work by Whipple[10] showed that active substances were found in watery extracts, alcoholic extracts, and in the extracted liver residue. The sum of these three sources of regenerating power obtained from experimental anemic dogs was from sixty to seventy grams of hemoglobin over a two weeks' period. The inorganic ash of beef liver was likewise potent in their experimental anemias, but it only contained one-half the potent factors present in whole liver—Robscheit-Robbins.[11] This led to the conclusion that a group of substances present in whole liver were responsible for the increased hemoglobin response in experimental anemias. Following this, Whipple[12] obtained a fraction assaying 70 per cent of the potency of whole liver in producing hemoglobin regeneration on posthemorrhagic anemic dogs. The summary of the clinical reports shows that whole liver is more beneficial in the treatment of secondary anemias than Minot's extract (Middleton,[13] Dyke,[14] Powers,[15] Keefer[16]).

Minot and Murphy[17] concluded from their observations that liver contained a substance which was specific in the transformation of a megaloblastic bone marrow to a normoblastic one. The active principle in liver as yet has not been isolated, although certain characteristics and properties of the antianemic material have been determined. Cohn, et al.,[18] presumed from their chemical refractionation of the effective material that the active substance is in the nature of a nitrogenous base or polypeptid.

Robscheit-Robbins and Whipple[19] demonstrate in their earlier work that whole liver plus iron salts was more beneficial in the treatment of posthemorrhagic anemia than either substance when administered alone. On the combined feeding they were able to produce as high as 140 grams of hemoglobin per two weeks' period in their experimental anemic dogs. Keefer and Yang[16] from their clinical report of 37 cases of secondary anemia concluded that whole liver and iron given in combination were more effective than either given alone. This was further substantiated by the clinical reports of other investigators.

The value of inorganic elements other than iron in the treatment of anemias has received widespread interest. Hart, Steenbock, and their coworkers[20] demonstrated regeneration of hemoglobin by the addition of small amounts of copper to nutritional anemic rats which were on minimal amounts of pure iron, inadequate to produce hemoglobin regeneration. Elvehjem, et al.,[21] have recently shown that a trace of copper is necessary as a catalyst for inorganic iron in the construction of the hemoglobin molecule. Satisfactory clinical results were reported by Lewis,[22] Josephs,[23] and Mills,[24] on the administration of iron and copper in the treatment of nutritional and idiopathic anemia in infancy, childhood, and adults. The summary of the current literature as to the value of germanium, arsenic, manganese, nickel, cobalt, and other inorganic elements in the regeneration of hemoglobin leaves the matter still in doubt.

Our work was undertaken to determine the value of a mixture of an aqueous concentrated liver extract, neutral glycerol-iron, and blood, in the treatment of secondary anemias. Preliminary studies in posthemorrhagic anemic dogs gave promising results.

THEORY AND METHODS

The liver fraction used was an aqueous extract from fresh horse livers, unpurified and evaporated in vacuo, so that one part of the extract represented four parts of liver. The fraction active in secondary anemia, as described by Whipple[12] was present in this extract. One of us, Meyer,[25] was able to demonstrate greater hemoglobin regeneration on the administration of this aqueous concentrated liver extract to posthemorrhagic anemic dogs than the equivalent amount of the 70 per cent alcohol-soluble liver fraction containing the active "antipernicious anemia" principle.

Ferrous chloride was added to the above concentrated aqueous liver extract as a neutralized glycerol solution, which was incorporated in defibrinated blood serum. It may be concluded from the reports of Starkenstein[26] and Reimann and Fritsch[27] that ferric compounds are less readily absorbed than the bivalent iron. The soluble iron salts are transformed into chlorides in the stomach, and partially into hydroxides or carbonates when coming in contact with the alkaline pancreatic juice, and in this way their absorption is hindered. It was thought that a mixture of ferrous chloride and glycerol might be of greater value than ferrous chloride alone, since glycerol prevents the precipitation of iron by alkali.

In the preparation of this compound, a concentrated solution of ferrous chloride was mixed with an excess of glycerol and the reaction of the mixture was adjusted to P_H 6.8. The object of this partial neutralization was to make it less astringent to the mucosa of the stomach. This neutralized glycerol-iron solution is not perfectly stabile, when diluted with water. A precipitate is apt to be formed on longer standing. However, it was found that serum acts as a protective stabilizing colloid, and that the presence of the formed blood elements does not interfere with this effect. In using defibrinated blood for this purpose, one also introduces the possible additional benefit of hemoglobin. In order to determine the relative effect on hemoglobin regeneration of this neutral glycerol-iron compound, equivalent amounts of iron solutions in the form of aqueous ferrous chlorides and acid glycerol-iron were administered to "control" groups of experimental anemic dogs. Although comparatively good responses were obtained in all three groups, greater and more consistent hemoglobin regeneration was noted in the group on the neutralized glycerol-iron compound.[25]

The ineffectiveness of hemoglobin in anemic animals found by Abderhalden[28] was probably due to the rather low iron content (.336 per cent), which was evidently insufficient to produce hemoglobin regeneration. It seems, therefore, that the administration of blood in large enough quantities to supply the desired amount of iron is impractical. Even though the effective value of hemoglobin as a source of iron is limited, the organic component might play a certain rôle. It is obvious that the pyrrol complex of porphyrin may be formed in different ways if it should not be present in the food in sufficient quantities. But just as the assimilation of iron can be impaired, it is possible that the synthesis of porphyrin may not be performed satisfactorily in certain anemias. In such cases of anemia the porphyrin part of hemoglobin may be just as important as the iron. An addition of inorganic iron in a suitable form may be of benefit in making the hemoglobin more effective.

Theoretically a combination of concentrated aqueous liver extract and the neutralized glycerol-iron compound in defibrinated blood includes all the possible known factors concerned in hemoglobin regeneration. The posthemorrhagic anemic dogs receiving this combined mixture showed a greater and more uniform erythrocyte and hemoglobin response than the groups on the various individual components.[25]

Syrup and flavoring agents were added to improve the taste and alcohol as a preservative (14½ per cent). This mixture is designated as preparation Bl 105 in this report. A careful analysis of one and one-half ounces of this preparation, which was the daily average dose administered to our cases, shows that it contains the extract of 84.4 grams of whole liver, a total of 104.24 mg. of metallic iron (6.75 mg. from liver; 5.62 mg. from hemoglobin; and 91.87 mg. from the neutral glycerol-iron compound) and a total of 1.4 mg. of metallic copper.

A comparative study was made by treating a series of patients with secondary anemia as "controls" on a known "weak" preparation, Bl 101. One and one-half ounces of this preparation, which was the amount administered daily, contained liver broth expressed from 16.8 grams of boiled liver, 10.6 mg. of iron, and traces of copper, to which the same amounts of flavoring agents and alcohol was added as in Bl 105.

The clinical work was made possible through the courtesy and cooperation of the Cook County Hospital Staff. The cases reported were patients with secondary anemias of the common variety occurring in a large institutional practice. No attempts were made in selecting ideal cases. The patients were accepted routinely as they entered the hospital, irrespective of the type or seriousness of their malady. Proper medical and surgical care was immediately instituted, whenever necessary. Most of these patients received a well-balanced diet, with the exception of a few patients who required special dietary management. Complete blood examinations including reticulocyte counts were made at one- to five-day intervals during the period of hospitalization, and at two-week intervals in a follow-up clinic when discharged.

It is obvious that in most instances individual study is necessary, as each patient presents a complex picture of various causative factors for the anemia, which is seldom identical in two patients with the same disease. For this reason the types of secondary anemias under observation in the "treated" groups were only classified according to their "chief" etiological causes. A total of 112 cases have been studied.

RESULTS

The first twenty-nine patients under observation with secondary anemia were given one and one-half ounces of the known "weak" preparation (Bl 101) daily. Because of the limited number and diversity of cases in this series, the results obtained were tabulated under one group, Table I. Moderate improvement was observed in Cases 4, 8, and 20, which was undoubtedly due to the usual hematopoietic response following acute hemorrhages. It is quite evident from the summary of this series, showing an average daily gain of .034 per cent hemoglobin and 2,500 red blood cells, that the hematologic response was only slightly influenced by the "weak" preparation (Bl 101) or by the specific surgical or

medical therapy during the period of observation. Because little or no progress was made on this preparation by Patients 10 and 20 to 29 (inclusive), the treatment was changed to the more potent preparation Bl 105 following which, with the exception of Cases 26 and 28, the hemoglobin and the red blood cell count became normal. Case 26, an inoperable gastric carcinoma, showed a moderate response, while Case 28, a bacterial endocarditis, showed no response to either preparation.

The results obtained from the administration of one and one-half ounces daily of Bl 105, the concentrated liver-iron and hemoglobin mixture, to patients with various types of secondary anemia, are recorded in Tables II, III, IV, IV-A, V, and VI.

The cases classified under Table II were of the acute hemorrhagic type and include diseases associated with frank hemorrhage which was the predominating cause of the anemia. The diseases primarily responsible for the acute anemia in this group consisted of: incomplete abortions; ruptured ectopic pregnancies; placenta previas; a postpartum hemorrhage; and a bleeding peptic ulcer. It is obvious that stopping the source of hemorrhage is the most important factor in recovery. It also seems logical that the administration of a concentrated preparation supplying in an easily assimilated form the necessary blood-building elements plus the addition of a hemopoietic stimulant may shorten the period of blood regeneration and convalescence. Treatment was started as soon as permissible, each patient receiving one-half ounce of preparation Bl 105 three times a day. In many instances moderate bleeding continued for several weeks while on therapy. The best gains were observed in this series, showing an average daily gain of .537 per cent hemoglobin and 30,538 red blood cells. The additional increase is probably explainable by the acute short hemorrhages being insufficient to deplete the body of the blood-building elements and the good response of the preexisting healthy bone marrow.

Patients with histories of moderate or profuse bleeding, the duration of which was from one month to several years, were classified under Table III as chronic posthemorrhagic anemias. The diseases most commonly met with in these cases were bleeding fibroids, bleeding hemorrhoids, and bleeding peptic ulcers. In most instances surgical treatment was necessary to eradicate the source of hemorrhage, with the exception of the patients with bleeding peptic ulcers, who were placed on Sippy's management. Each patient received one-half ounce of the more concentrated whole-liver-iron preparation Bl 105 t.i.d., which was instituted shortly after their admittance to the hospital. Of the twenty-nine patients treated in his series, twenty-three, with an initial average of 40 per cent hemoglobin and 2,420,000 red blood cells per cubic millimeter, became normal during an average period of 75.3 days, with an average daily response of .541 per cent hemoglobin and 24,435 red blood cells.

Although the results in this series of patients resemble those obtained in the anemias which followed acute hemorrhages, the somewhat smaller response can probably be attributed to the long period of continuous bleeding with the resultant depletion of the blood-building elements, and an overstimulated and fatigued hematopoietic system. Moderate continuous bleeding persisted in several patients, ten in this series, while on treatment, and, although a normal hemo-

TABLE I

CASES OF SECONDARY ANEMIA FROM VARIOUS CAUSES TREATED ON KNOWN WEAK PREPARATION Bl 101 AS "CONTROL" ½ OUNCE T. I. D.

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%)		CHANGES IN R.B.C. IN MILLIONS		WEIGHT CHANGES POUNDS	COMMENTS
						BEFORE	AFTER	BEFORE	AFTER		
1	F	29	Incomp. abortion. Congenital heart	15 da.	28	45	38	2.01	2.46		No prog.*
2	F	41	Bleed. fibroids	1 yr.	24	34	40	2.78	3.07		Hysterectomy*
3	M	30	Osteomyelitis of elbow joint	2 mo.	46	52	58	3.07	3.41	120-145	Drainage
4	F	28	Incomp. abortion	1 mo.	35	41	47	1.82	2.31	107-109	Curettage*
5	M	31	Bact. endocarditis	6 mo.	36	37	27	2.45	1.78		Progressively downhill. Went home
6	F	27	Incomp. abortion	11 da.	30	39	39	2.94	2.74	119-123	Placenta removed
7	F	34	Salpingitis. Pelvic peritonitis	2½ yr.	55	53	58	3.93	3.26		Bilateral salpingectomy. Temp. continued several weeks. Post-operative*
8	M	60	Bleed. gastric ulcer	10 da.	20	57	69	2.60	3.88		Also on Sippy management. Transferred from hospital
9	F	30	Postoperative thrombophlebitis	10 da.	17	29	36	2.04	3.14		Low grade temp. throughout*
10#	F	29	Incomp. septic abortion	1 wk.	69	46	45	2.10	3.12		Placed on Bl 105. Normal in 91 da. Table IV-A, Case 10
11	M	47	Banti disease	1 yr.	57	52	61	2.52	3.03	143-150	*
12	F	23	Septic abortion	4½ mo.	34	38	48	2.50	2.37		*
13	M	35	Empyema	10 da.	27	63	63	3.14	3.78		Rib resection. Drainage*
14	F	20	Incomp. septic abortion	3 da.	16	40	53	2.73	3.20		Had 450 c.c. blood transfusion 2 days prior*
15	F	26	Bilateral salpingitis. Pelvic peritonitis	6 wk.	34	68	45	2.79	2.57	131-134	Bilateral salpingectomy*
16	M	15	Encapsulated empyema	4 wk.	33	39	43	2.59	2.34		Rib resection. Drainage*
17	F	38	Comp. abortion	3 wk.	68	45	51	2.49	3.29	147-146	Curettage*

TABLE I—CONT'D

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%)		CHANGES IN R.B.C. IN MILLIONS		WEIGHT CHANGES POUNDS	COMMENTS
						BEFORE	AFTER	BEFORE	AFTER		
18	M	13	Septic 2nd deg. burns	2 mo.	67	64	62	3.67	3.09		*
19	F	36	Ectopic preg. ruptured	4 da.	14	52	47	2.92	2.62	171-174½	Salpingectomy. 400 c.c. blood given 3 da. prior to treatment
20#	F	21	Ruptured ectopic preg.	1½ da.	49	36	65	2.19	2.72		Changed to Bl 105. Table II, Case 1, Comp.
21#	F	28	Pelvic peritonitis. Thrombophlebitis following hysterectomy	5 mo.	67	60	65	3.55	3.60		Table IV, Case 3, Comp.
22#	F	19	Rheumatic heart disease	3 wk.	67	29	43	2.95	3.38	109-115	Table IV, Case 4, Comp.
23#	M	67	Decompensated heart. Idiopathic anemia	4 yr.	16	25	28	1.81	2.01		Table IV, Case 7, Comp.
24#	F	40	Bleed. fibroids	2 yr.	63	34	37	3.09	3.12		Hysterectomy. Table III, Case 9, Comp.
25#	F	43	Hydatiform mole	3 mo.	44	39	41	2.13	2.96		Hysterectomy. Table III, Case 10, Comp.
26#	M	63	Ca of stomach	2 yr.	79	31	19	1.10	1.73		Table V, Case 7
27#	F	39	Bleed. fibroids	2½ mo.	25	49	56	3.42	2.79		Hysterectomy. Table III, Case 13, Comp.
28#	F	44	Bact. endocarditis	1 yr.	65	42	44	2.83	2.28		Table IV, Case 16, Incomp.
29#	F	27	Purpura hemorrhagica	4 yr.	92	62	73	4.19	3.60		Table IV, Case 2, Comp.
			Total		1276	1301	1348	80.35	83.66		
			Average Patient		44	44.9	46.4	2.77	2.88		
			Average Net Gain			1.5		0.11			
			Average Daily Gain			0.34% Hb. (Sahli)		2500 R.B.C.			

F Indicates female. M Indicates male. *Cases that left hospital and did not return for further observation. # Changed to Bl 105.

SECONDARY TABLE II

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIA OF THE ACUTE POSTHEMORRHAGIC TYPE TREATED WITH PREPARATION BL 105 ½ OUNCE T.I.D. (RECOVERY COMPLETE)

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION DAYS	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%) BEFORE	HEMOGLOBIN SAHLI (%) AFTER	R.B.C. IN MILLIONS BEFORE	R.B.C. IN MILLIONS AFTER	WEIGHT CHANGES POUNDS	COMMENTS
1	F	21	Ectopic pregnancy	1½	60	65	86	2.72	4.35	99-113½	Salpingectomy†
2	F	29	Incomp. abortion	12	47	28	80	1.23	4.00	147-151	Curettage
3	F	40	Incomp. abortion	21	88	62	82	2.76	4.43	107-118	Placenta expressed
4	F	20	Retained placenta	14	106	54	81	2.43	4.25	99-100	Curettage # 1
5	F	26	Ruptured ectopic pregnancy	1	44	58	84	2.70	4.18	145-150	Salpingectomy
6	F	38	Ruptured ectopic pregnancy	½	44	50	81	2.30	4.13	199-199	Salpingectomy
7	M	49	Bleeding peptic ulcer	21	63	31	81	1.99	4.18	155-184	Gastroenterostomy
8	F	29	Incomp. abortion	½	94	57	86	2.90	4.85	139-135	Curettage
9	F	27	Incomp. abortion	1	42	47	83	2.94	4.53	101-104	Curettage # 2
10	F	27	Ectopic pregnancy	½	44	39	81	1.92	4.32	113-115	Salpingectomy # 3
11	F	21	Incomp. abortion	32	83	36	75	1.81	4.08	149-150	Expelled placenta
12	F	36	Incomp. abortion	22	87	25	83	1.29	4.12	120-132	Also had rectal bleeding
			Total		802	552	983	27.05	51.42		
			Average Patient		66.8	46	81.9	2.25	4.29		
			Average Net Gain			35.9		2.04			
			Average Daily Gain			0.537% Hb. (Sahli)		30,538 R.B.C.			

*SIMILAR CASES STILL ON TREATMENT (NOT COMPLETED)

13	F	24	Incomp. abortion	20	17	19	60	1.61	3.46		Expelled placenta
14	F	26	Postpart. hemorrhage	3	86	18	77	0.91	4.05		Moderate temperature throughout treatment # 4
15	F	25	Postpart. hemorrhage	3	46	53	70	2.59	3.88		Moderate temperature
16	F	28	Placenta previa	½	29	31	70	1.38	3.42		Also had breast abscess
17	F	30	Placenta previa	3	29	42	53	2.27	3.25		Labor terminated
18	F	35	Incomp. abortion	14	16	18	25	1.08	1.49		Curettage
19	F	33	Incomp. abortion	7	37	40	71	2.26	3.93		Moderate temperature
20	F	26	Incomp. abortion	7	16	24	45	1.66	3.17		Placenta expelled

*Average daily Hb. and R. B. C. gain calculated on complete cases only.

F Indicates female. M Indicates male. †No response in 49 days to Bl. 101. # 1 400 c.c. blood given 2 wks. prior to treatment. # 2 500 c.c. blood given 10 days prior to treatment. # 3 450 c.c. blood transfusion 2 days prior to treatment. # 4 540 c.c. blood transfusion at beginning of treatment.

globin and red blood cell count was produced, this was also probably a contributing factor in producing a lower average daily gain. Cases 9, 10, and 13 (Table III) previously treated with the "weak" preparation (Bl 101) and showing little progress, became normal on treatment with the more concentrated preparation (Bl 105).

The secondary anemias in Table IV treated with preparation Bl 105 showed an average daily gain of .3 per cent hemoglobin and 16,400 red blood cells in the completed series, and .22 per cent hemoglobin and 10,700 red blood cells in the incompleted group. Ten of the twenty-four patients in this group became normal on treatment during an average of 101.6 days. Patients who failed to return to the clinic, and those who are still under observation, are classified in the incompleted group. Many etiologic and contributing factors entered into the production of the anemia in this group, which included patients primarily with infectious, toxic, and dietary diseases.

Very little response was obtained in Cases 13, 16, 17, 23, and 20 of the incompleted group, which consisted of a terminal glomerular nephritis, a bacterial endocarditis, two cases of far-advanced tuberculosis, and one of Hodgkin's disease running a septic course. Although slow progress was made in the cases with low-grade septic processes, the hemoglobin and erythrocyte count became normal after long-continued treatment on preparation Bl 105. Case 7 in Table IV, a young female with a congenital splenic anemia, gave a history of eating liver and taking liver extracts for the past five years, and, although moderate improvement was noted, she at no time felt well enough to carry on her daily activities. This patient was started on preparation Bl 105 following splenectomy. The splenectomy was probably the fundamental factor in her complete recovery, although it was interesting to note that there was a moderate drop in the hemoglobin and the erythrocyte count dropped when taken off of treatment during an interval of 49 days (Table VI, Case 22).

The patients classified under Table IV-A, treated with whole-liver-iron preparation, showed an average daily gain of .434 per cent hemoglobin (Sahli) and 18,300 red blood cells. We believe that the data obtained from these eleven cases constitutes the best evidence that the concentrated liver-iron preparation (Bl 105) was really effective in improving the anemia. In these cases we are of the opinion that the improvement may only be accounted for as being the result of the whole-liver-iron therapy. Although we believe that a number of the other patients recorded in the tables were benefited by the concentrated liver-iron preparation, their improvement may be accounted for in other ways.

It is interesting to note the good response of Cases 1 and 6 to preparation Bl 105. Both patients were white women, about forty years of age, giving a history of long continuous rectal bleeding which came on only before and during each menstrual period. A long dietary deficiency history was also obtained in both instances. Their chief complaints were: weakness, pallor, loss of appetite, and occasional gastrointestinal disturbances. Careful gastrointestinal study and numerous proctoscopic examinations failed to reveal the source of bleeding, which continued throughout the entire course of treatment. The essential findings in both patients were an achylia gastrica and a high-grade secondary anemia, with a low-color index and leucopenia, of the chronic "chlorotic" type first described by

SECONDARY TABLE III

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIAS OF THE CHRONIC POSTHEMORRHAGIC TYPE TREATED WITH PREPARATION BL 105 ½ OUNCE T.I.D. (RECOVERY COMPLETE)

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%)		CHANGES IN R.B.C. IN MILLIONS		WEIGHT CHANGES POUNDS	COMMENTS
						BEFORE	AFTER	BEFORE	AFTER		
1	M	54	Hypertension and bleeding peptic ulcer	30 da.	74	42	72	1.77	3.55	131-138	Also on Sippy treatment
2	F	29	Marg. placenta previa	42 da.	49	33	89	1.65	4.40	154-160	Labor terminated by bag induction
3	F	38	Bleeding fibroids	2 yr.	118	45	80	2.64	4.24	142-162	Hysterectomy
4	F	36	Bleeding fibroids	1 yr.	90	41	81	2.20	4.95	120-132	Hysterectomy
5	F	38	Bleeding fibroids and pregnancy	7 mo.	125	59	86	3.40	4.24	128-136	First operation exploratory. Preg. removed; second hysterectomy
6	F	44	Bleeding fibroids	1 yr.	77	42	91	3.72	4.24	155-160	12 x-ray treatments 1 yr. ago; panhysterectomy prior to med.
7	M	49	Bleeding peptic ulcer	1 mo.	69	27	55	2.04	3.20	148-163	Also on Sippy management. Did not return to hospital.
8	F	43	Bleeding fibroids	3 yr.	55	60	85	2.78	4.11	126-135	Hysterectomy # 1
9	F	40	Bleeding fibroids	2 yr.	73	37	82	3.12	4.28	122-122	Hysterectomy followed by large stitch abscess†
10	F	43	Hydatiform mole	3 mo.	95	41	85	2.96	4.42	95- 99	Hysterectomy; 4 x-ray treatments while on treatment‡
11	M	40	Bleeding hemorrhoids	6 mo.	60	44	81	3.18	4.58	189-206	Hemorrhoidectomy
12	F	34	Incomp. abortion	3 mo.	59	57	82	2.83	4.13	111-110	Curettage
13	F	39	Bleeding fibroids and old double mitral lesion	6 mo.	74	56	83	2.79	4.37	118-120	Hysterectomy followed by stitch abscess§
14	M	36	Bleeding hemorrhoids, double mitral lesion. Cholelithiasis	13 yr.	77	16	90	1.10	4.56	117-131	Hemorrhoidectomy. Cholecystectomy
15	F	32	Bleeding fibroids	7 mo.	56	38	84	2.61	4.71	107-110	Hysterectomy # 2
16	M	27	Bleeding hemorrhoids	7 mo.	41	48	83	3.56	4.91	160-161	Hemorrhoidectomy
17	M	43	Bleeding hemorrhoids	3 yr.	67	41	80	2.57	4.49	165-171	Hemorrhoidectomy

SECONDARY TABLE III—CONT'D

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%)		CHANGES IN R.B.C. IN MILLIONS		WEIGHT CHANGES POUNDS	COMMENTS
						BEFORE	AFTER	BEFORE	AFTER		
18	F	46	Fibroid uterus	1 yr.	114	37	80	1.99	4.06	143-163	Hysterectomy
19	F	36	Incomp. abortion	3 mo.	58	45	77	2.18	4.10	129-134	Expelled placenta in hospital
20	F	31	Bleeding fibroids	1 yr.	98	20	86	1.19	4.30	96- 99	Hysterectomy preceded by radium treatment
21	M	45	Bleeding peptic ulcer	5 mo.	112	25	80	1.15	4.29	130-145	Also on Sippy management. Mod. bleeding during first half of treat.
22	F	32	Incomp. abortion	4 mo.	63	28	82	1.15	4.10	126-127	Curettage
23	M	38	Bleeding peptic ulcer	6 wk.	29	59	83	2.97	4.23	152-155	Also on Sippy management
			Total		1733	939	1877	55.56	98.46		
			Average Patient		75.3	40.8	81.6	2.42	4.26		
			Average Net Gain			40.8		1.84			
			Average Daily Gain			0.541% Hb. (Sahli)		24,435 R.B.C.			
			*SIMILAR CASES STILL ON TREATMENT (NOT COMPLETE)								
24	F	47	Bleeding fibroids	6 mo.	72	23	74	1.67	3.97	187-195	Hysterectomy
25	F	31	Bleeding fibroids	1 mo.	38	37	57	2.11	3.73		Hysterectomy
26	M	38	Bleeding hemorrhoids	2 yr.	72	29	61	2.67	3.75	192-204	Hemorrhoidectomy # 3
27	F	31	Bleeding fibroids	5 yr.	57	29	70	1.91	3.93	163-175	Hysterectomy # 3
28	M	31	Spondylitis. Bleeding peptic ulcer and old mitral insuff.	1 yr.	38	30	59	2.58	4.31		Mod. bleeding while on treatment. Also on Sippy management # 4
29	F	36	Bleeding peptic ulcer	1 mo.	43	38	56	2.76	4.06		Also on Sippy management

*Average daily Hb. and R. B. C. Gain calculated on complete cases only. F indicates female. M indicates male. # 1 Two 500 c.c. blood transfusions given 2 months prior to medication. †On Bl 101 for 2 months. No progress. ‡No progress on Bl 101 in 14 days. # 2 250 c.c. blood transfusion given prior to treatment. # 3 Did not return for further treatment. # 4 Made no progress in 3½ weeks prior to medication. §No progress on Bl 105 in 25 days.

TABLE IV

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIAS RESULTING FROM INFECTIOUS, TOXIC, AND DIETARY DISEASES (RECOVERY COMPLETE) Treated with Preparation Bl 105 ½ Ounce T.I.D.

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%)		CHANGES IN R.B.C. IN MILLIONS		WEIGHT CHANGES POUNDS	COMMENTS
						BEFORE	AFTER	BEFORE	AFTER		
1	M	52	Pellagra. Polyneuritis	6 mo.	102	57	84	2.50	4.33	146-170	Nervous symp. improved. Able to carry on normal activities.
2	F	27	Achondroplastic dwarf. Anemia of pregnancy	5 mo.	94	51	83	2.44	4.04	84- 86	Cesarean section. On treatment at end of preg. and following surg.
3	F	22	Incomplete septic abortion	1 mo.	112	50	81	2.69	4.87	104-118	Curettage. Febrile throughout therapy and profuse vaginal bleed.
4	F	23	Incomplete septic abortion	3 mo.	90	29	80	1.62	4.42	118-120	Curettage†
5	F	25	Preg. subcutaneous abscess	4 wk.	65	47	82	2.22	4.22	101-111	Abscess drained 1 mo. prior to delivery when treat. was started
6	F	37	Incomp. abortion. Generalized peritonitis pelvic abscess	3 mo.	181	66	83	2.72	4.10	118-173	Colpotomy. Septic course throughout
7	F	18	Congenital splenic anemia	5 yr.	57	59	83	3.58	4.53	134-127	Splenectomy 2 wk. prior to therapy
8	F	39	Incomp. septic abortion	7 da.	189	43	83	2.23	4.14		Low grade temp. throughout
9	F	42	Chronic infectious anemia	3 yr.	41	73	86	3.97	4.89		# 1
10	F	29	Incomp. septic abortion	1 wk.	91	46	81	3.12	4.31		No response to Bl 101 in 69 da. Table 1, Case 10
			Total		1016	512	823	27.18	43.82		
			Average Patient		101.6	51.2	82.3	271	438		
			Average Net Gain			31.1		1.66			
			Average Daily Gain			0.3% Hb. (Sahli)		16,400 R.B.C.			

TABLE IV—CONT'D

SIMILAR TYPES—TREATMENT NOT COMPLETED

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%)		CHANGES IN R.B.C. IN MILLIONS		WEIGHT CHANGES POUNDS	COMMENTS
						BEFORE	AFTER	BEFORE	AFTER		
11	F	37	Incomp. septic abortion. Pelvic peritonitis	8 da.	30	54	55	2.80	3.38		Temp. throughout colpotomy and salpingectomy several wks. prior to treat. # 2
12	F	48	Bron. pneumonia empyema & bleed. hemorrhoids	2 mo.	73	27	49	1.88	3.04		Septic temp. for 57 days. # 2
13	M	16	Glomerular nephritis subacute attack	6 yr.	67	38	38	1.93	2.08		Died in uremia. No prog. on Bl 105
14	F	29	Malaria	3½ mo.	32	26	66	1.13	2.95		Also received 20 gr. quinine daily. # 2
15	F	35	Pelvic peritonitis	1 yr.	125	45	62	2.62	3.41	107-110	Septic temp. throughout. Etio. not determined. Little prog.
16	F	44	Bact. endocarditis	1 yr.	194	44	56	2.28	2.90	142-130	No improvement
17	M	34	T. B. scapula. Mult. abscesses	4 mo.	56	52	70	2.56	3.73		End of therapy. Trans. to T. B. hosp.
18	F	25	Anemia of pregnancy and post-partum hemorrhage	8 mo.	134	47	73	2.96	4.44	133-136	On treat. during preg. had severe postpart. hemorrhage while on theraphy. # 2
19	F	22	Incomp. septic abortion	4 mo.	40	30	59	2.14	3.52		Still running septic temp.*
20	M	16	Hodgkin's disease	10 yr.	23	46	43	2.83	2.94		Septic temp. no prog.*
21	F	41	Puerperal sepsis	15 da.	64	27	67	1.58	3.40	163-174	Started on treat. 18 days following delivery*
22	F	16	Unresolved pneu.	5 wk.	28	61	64	3.05	3.25		Temp. for 2 wk. while on treat. no progress*
23	M	35	T. B. spine	1 yr.	81	46	46	3.33	3.32		Bedridden. No prog. although bl. level main.
24	F	39	Septic abortion	21 da.	90	40	68	1.85	3.56	151-150	Recurrent low-grade temp. profuse and irregular menses. # 2
			Total		1047	593	816	35.24	46.25		
			Average Patient		74.7	42.3	58.3	2.51	3.30		
			Average Net Gain			16.0		0.79			
			Average Daily Gain			0.22% Hb. (Sahli)		10,700 R.B.C.			

F Indicates female. M Indicates male. †400 c.c. blood transfusion 1 week prior to treatment. # 1 Very little progress to whole liver and the alcohol soluble liver extract, prior to present medication. # 2 Cases that did not return for further observation or treatment. *Cases still on treatment and observation.

TABLE IV-A

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIAS OF THE "CHLOROTIC," IDIOPATHIC, TOXIC, AND INFECTIOUS TYPE. (RESPONSES PRIMARILY ATTRIBUTED TO THE WHOLE-LIVER-IRON THERAPY)

TREATED WITH PREPARATION BL 105 ½ OUNCE T. I. D.

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%)		CHANGES IN R.B.C. IN MILLIONS		WEIGHT CHANGES POUNDS	COMMENTS
						BEFORE	AFTER	BEFORE	AFTER		
1	F	41	Chlorotic type of anemia. Frequent rectal bleeding	16 yr.	104	49	85	3.08	4.16	130-145	Had achylia. # 1
2	F	27	Purpura hemorrhagica	4 yr.	65	73	80	3.60	4.28		Bruised easily. No hemorrhages while on treatment. # 2
3	F	28	Pelvic peritonitis. Thrombophlebitis following hysterectomy	5 mo.	73	65	83	3.60	4.38	108-119	Febrile throughout treatment
4	F	19	Rheumatic heart disease	3 wk.	65	43	85	3.38	4.59	109-115	Mod. temp. throughout treat. # 3
5	M	37	Acute lead pois. Quadriplegia	2 mo.	45	53	73	3.27	4.87		End of treat. transferred
6	F	39	Chlorotic anemia also frequent rectal bleeding	3 yr.	62	43	84	3.66	4.24	114-112	Had achylia. # 4
7	M	67	Decompensated heart. Idiopathic anemia	4 yr.	227	28	80	2.01	4.21	162-173	Digitalized. Active at end of treatment
8	F	24	Syphilis. Arsphenamine react. (Dermatitis & Hepatitis)	7 da.	84	25	78	2.16	3.83	98-101	Liver still enlarged*
9	F	44	Decompensated heart. Idiopathic anemia	5 yr.	54	13	59	1.28	4.04		Kept digitalized. Made good prog.*
10	M	59	Decompensated heart	1 yr.	27	36	60	2.16	3.12		Digitalized*
11	M	23	Ulcerative colitis	2 yr.	52	44	76	2.63	4.93	133-160	*
			Total		862	472	843	30.83	46.65		
			Average Patient		78.3	42.8	76.6	2.80	4.24		
			Average Net Gain			34.0		1.44			
			Average Daily Gain			.434% Hb. (Sahli)		18,300 R.B.C.			

F Indicates female. M Indicates male. # 1 No response to Fraction G extract in 30 days. # 2 Highest count in 4 years of observation. # 3 Made little progress on Bl 101 in 67 days. # 4 No response to Fraction G liver extract in 1 month therapy. *Cases still on treatment and observation.

Faber. No response was obtained from the administration of the 70 per cent alcohol-soluble liver extract during one month's treatment. The hemoglobin and erythrocyte count became normal in both patients when changed to the concentrated liver-iron preparation. Cases 7, 10, and 11, three patients with marked cardiac decompensation and severe secondary anemia of unexplained etiology, responded well to preparation Bl 105. The occurrence of this disease during the later years of life, with the history of recurrent attacks of cardiac decompensation and the associated anemia, which complicated this picture only after several years, led us to believe that it was probably due to a dietary deficiency based on the gastrointestinal disturbances associated with the frequent attacks of cardiac failure.

Table V included a number of patients with far-advanced inoperable carcinomas with severe secondary anemia who were treated with preparation Bl 105. A moderate increase in hemoglobin and red blood cells was noted in Cases 1, 3, 7, and 8. Although little or no progress was observed in most instances, the original blood level at the beginning of treatment remained fairly stationary up to the time of exitus.

In addition to the beneficial hematologic response obtained in the largest majority of patients on treatment, other noteworthy effects of the liver-iron and hemoglobin preparation were observed. The appetite was improved. There was also a corresponding increase of strength, weight, and in feeling of well-being. It was surprising to observe these changes, occasionally, in several of the inoperable carcinomatous patients. The period of preoperative preparation in undernourished anemic patients with low-grade septic processes was definitely shortened, as well as the duration of convalescence from infectious diseases and operative procedures.

The summary of the reticulocyte response observed in the ninety-four cases in the "treated" groups treated with Bl 105, showed that the most frequent rise was from 1.5 to 4.5 per cent, although in many instances a rise of from 8 to 10 per cent was noted. This excluded the reticulocyte average in the carcinomatous group with bone-marrow metastasis, where a response as high as 18.6 per cent (Case 4) was observed, produced probably by the irritation of the hematopoietic system by the metastasis.

Definite hematologic and clinical improvement as previously stated was observed in the largest majority of patients treated with the concentrated liver-iron and hemoglobin preparation. However, insuperable difficulties were encountered in evaluating the effectiveness of this preparation as a hemopoietic in the various types of secondary anemias treated. This can readily be seen when one considers the many complexities that entered into the pathogenesis of these anemias. This was further complicated during treatment because in many instances specific medical and surgical treatment was necessary. As yet no reliable biological tests have been discovered to evaluate the effect of such a preparation of liver-iron and hemoglobin other than its effect upon the regeneration of blood in experimental uncomplicated anemias.

Although we were impressed by the effectiveness of the concentrated liver-iron and hemoglobin preparation Bl 105 in the largest majority of "treated" cases as compared to the "control" group receiving a known weak formula, and

SECONDARY TABLE V

RESULTS OBTAINED IN CASES OF SECONDARY ANEMIAS IN CASES OF MALIGNANCY TREATED WITH PREPARATION BL 105 ½ OUNCE T. I. D.

CASE	SEX	AGE	DIAGNOSIS	PROBABLE DURATION	DAYS ON TREATMENT	CHANGES IN HEMOGLOBIN SAHLI (%)		CHANGES IN R.B.C. IN MILLIONS		COMMENTS
						BEFORE	AFTER	BEFORE	AFTER	
1	F	48	Inoperable Ca of cervix	1 yr.	90	28	48	1.55	2.42	5 x-ray treat. mod. vag. bleed.*
2	M	69	Ca of prostate. Mult. bone metastasis	6 mo.	17	35	33	1.40	1.37	Patient died
3	F	30	Metrhg. Inoperable Ca of cervix	8 yr.	86	27	60	3.18	3.36	2 x-ray treat.†
4	M	57	Ca of stomach. Mult. bone metastasis	7 wk.	48	21	16	.98	.72	Patient died
5	M	76	Ca of stomach with bleed. & liver metastasis	1 yr.	78	30	27	2.40	1.96	†
6	M	65	Ca of stomach	2 yr.	35	43	47	2.70	2.83	Patient died
7	M	63	Ca of stomach	2 yr.	128	19	40	1.72	2.24	Patient died from gastric hemorrhage
8	M	70	Ca of stomach	6 mo.	28	24	55	1.13	2.93	*
9	M	40	Myosarcoma of chest wall	6 yr.	11	39	34	2.75	2.72	Removed tumor mass
10	F	43	Mult. myeloma	6½ mo.	54	33	33	1.62	1.42	Patient died
			Total		575	299	393	19.43	21.97	
			Average Patient		57.5	29.9	39.3	1.94	2.20	
			Average Net Gain			9.4		.26		
			Average Daily Gain			.16% Hb. (Sahli)		4,521 R.B.C.		

*Cases still on treatment. †Did not return for further observation. F Indicates female. M Indicates male.

TABLE VI

BLOOD CHANGES OCCURRING IN PATIENTS WHO HAVE BEEN OFF OF TREATMENT FOR VARIOUS PERIODS OF TIME, PREVIOUSLY TREATED WITH PREPARATION BL 105 AND DISCHARGED IMPROVED

CASE	SEX	AGE	REFERENCE TO PREVIOUS TREATMENT TABLE/CASE	INTERVAL OFF TREATMENT (DAYS)	CHANGES IN					COMMENTS	*See footnote		
					HEMOGLOBIN (SAHLI) %		R.B.C. IN MILLIONS		WEIGHT CHANGES POUNDS		DAYS ON TREATMENT	HB %	R.B.C. IN MILLIONS
					BEFORE	AFTER	BEFORE	AFTER					
1	F	21	T. 2 C. 1	182	86	82	4.35	4.03	113-127	No complaints	—	—	—
2	F	40	T. 2 C. 3	49	83	74	4.53	4.06	118-130	No complaints	37	81	4.12
3	F	20	T. 2 C. 4	182	81	66	4.25	3.39	100- 95	Pain in lower left quadrant. Appetite poor	36	78	4.00
4	F	26	T. 2 C. 5	139	84	84	4.18	4.01	150-146	No complaints	—	—	—
5	F	38	T. 2 C. 6	139	81	64	4.13	3.74	199-203	No complaints*	40	71	3.65
6	M	49	T. 2 C. 7	154	81	82	4.18	4.39	184-185	Ate liver 1 time a week	—	—	—
7	F	27	T. 2 C. 9	104	83	69	4.53	3.22	104-102	No complaints	14	81	4.01
8	F	29	T. 3 C. 2	139	80	65	4.40	3.85	160½-166	No complaints	Did not return		
9	F	38	T. 3 C. 3	149	80	78	4.24	3.81	162-170	No complaints	Did not return		
10	F	38	T. 3 C. 5	175	86	74	4.24	3.83	136-145	No complaints	35	80	4.26
11	F	43	T. 3 C. 8	182	85	70	4.11	3.93	135-137	No complaints	28	84	4.52
12	M	40	T. 3 C. 11	199	81	81	4.58	4.12	206-204	Eating ½ lb. liver t. i. d.	—	—	—
13	F	39	T. 3 C. 13	188	83	78	4.37	3.06	120-175	Complains of weakness	—	—	—
14	F	32	T. 3 C. 15	111	84	72	4.71	3.38	110-121	No complaints	28	80	4.12
15	M	27	T. 3 C. 16	78	83	84	4.91	4.10	161-165	No complaints	Did not return		
16	M	43	T. 3 C. 16	49	80	53	4.49	2.79	171-169	Rectal bleeding past 3 weeks	21	62	3.95
17	F	46	T. 3 C. 18	163	80	65	4.06	3.53	163-173	No complaints	56	81	3.59
18	F	31	T. 3 C. 20	64	86	89	4.30	4.26	99-105	Ate liver 3 to 4 times a week up to past 3 weeks	—	—	—
19	F	47	T. 3 C. 24	14	85	71	4.28	4.14	199-205	No complaints	40	82	4.21
20	M	52	T. 4 C. 1	203	81	75	4.33	2.82	170-182	No complaints	49	84	4.05
21	F	37	T. 4 C. 6	105	83	82	4.10	4.15	173-200	No complaints	—	—	—
22	F	18	T. 4 C. 7	49	83	74	4.53	4.06	127-134	No complaints	Did not return		
23	F	39	T. 4 C. 8	91	83	74	4.14	3.35	130-137	No complaints	28	80	4.05
24	F	40	T. 4 C. 9	77	86	78	4.89	3.81	176-176	Complains of generalized pains and aches	40	83	4.10
25	F	41	T. 4-A C. 1	167	85	76	4.16	4.07	145-147	Received ½ oz. Bl 105 daily during interval	25	80	4.55
26	F	28	T. 4-A C. 3	199	83	71	4.38	3.41	119-126	Pain in lower left quadrant. Swelling of left leg	14	77	4.16
27	F	19	T. 4-A C. 4	221	85	68	4.59	3.12	115-111	No complaints	Did not return		
28	M	67	T. 4-A C. 7	105	80	60	4.21	3.02	173-180	No complaints on digitalis	21	80	4.08

*The responses obtained in patients with recurrent anemias, after treatment was again instituted, are tabulated in the last three columns.

the good response obtained in eleven cases of secondary anemias classified under Table IV-A, which was primarily attributed to the administration of preparation Bl 105, conclusive evidence as to its effectiveness in blood regeneration in many of the secondary anemia patients was still lacking.

For this reason it was considered important to determine the hematologic changes, if any, that occurred in patients, when off of treatment at various intervals of time, previously "treated" and discharged with a normal hemoglobin and red blood cell count. Letters were sent to all of the patients, asking them to return to the clinic for a check-up. Of the twenty-eight patients who returned, the intervals off of treatment varied from fourteen to two hundred and twenty-one days. All types of secondary anemia previously classified in Tables II, III, IV, and IV-A were well represented in this group. Although noteworthy gains in weight were observed in most instances, twenty-one patients showed a definite drop in hemoglobin and red blood cells while off of treatment (Table V). All of these patients were in apparent good health except one (Case 16) who had frequent rectal bleeding during the prior three weeks, which was probably responsible for the recurrent anemia. The hemoglobin and red blood cell count remained normal in only seven of the twenty-eight patients. Three of these ate liver two to three times a week after having been discharged from the clinic.

Treatment with the concentrated liver-iron and hemoglobin preparation Bl 105 was again instituted in the sixteen patients with recurrent anemias, previously treated and discharged as normal. The increase in hemoglobin and erythrocytes (Table VI) obtained in fifteen of these sixteen patients after being treated again with preparation Bl 105 under home conditions, we feel is conclusive evidence of the effectiveness of this preparation in the regeneration of hemoglobin and red blood cells as there were no other factors to which their recovery could be attributed.

SUMMARY

One hundred and twelve patients with secondary anemia due to various causes have been treated with a preparation consisting of concentrated whole liver, iron in glycerol, and defibrinated blood. The rationale of the preparation used is discussed. A large percentage of this group of patients showed hematologic and clinical improvement on receiving the whole liver-iron preparation and other indicated therapy. We can only state with confidence that the improvement of eleven of the patients can be accounted for primarily on the basis of having received the whole-liver-iron preparation. Further evidence as to the effectiveness of this preparation was shown by the drop in hemoglobin and red cells when treatment was discontinued in twenty-one patients representing the various groups previously treated and discharged as normal. Hematologic recovery was obtained when treatment was again instituted under "home conditions." Our study has impressed us particularly in regard to the numerous and almost insurmountable difficulties inherent in the problem of determining clinically the value of a therapeutic agent in the hemorrhagic and idiopathic secondary anemias.

REFERENCES

1. Richter, O., Meyer, A. E., and Ivy, A. C.: The Treatment of Pernicious Anemia by Horse Liver Extract, J. A. M. A. **98**: 1623-1625 (May 7) 1932.

2. Meyer, A. E., Richter, O., and Ivy, A. C.: The Treatment of Pernicious Anemia With Equine Liver Extract Injectable Either Subcutaneously or Intravenously, Arch. Int. Med. In publication.
3. Cohn, E. J., Minot, G. R., Fulton, J. F., Ulrichs, H. F., Sargent, F. C., Weare, J. H., and Murphy, W. P.: The Nature of the Material in Liver Effective in Pernicious Anemia, J. Biol. Chem. **74**: 69 (July) 1927.
4. Giffin, H. Z., and Watkins, C. H.: Secondary Anemia, J. A. M. A. **95**: 587 (Aug.) 1930.
5. Castle, W. B.: Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia; The Effect of the Administration to Patients With Pernicious Anemia of the Contents of the Normal Human Stomach Recovered After the Ingestion of Beef Muscle, Am. J. M. Sc. **178**: 748-764 (Dec.) 1929.
6. Castle, W. B., and Townsend, W. C.: Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia of Beef Muscle After Incubation With Normal Human Gastric Juice, Am. J. M. Sc. **178**: 764-777 (Dec.) 1929.
7. Castle, W. B., Heath, C. W., Strauss, M. B., and Townsend, W. C.: The Relationship of Disorders of the Digestive Tract to Anemia, J. A. M. A. **97**: 904-907 (Sept.) 1931.
8. Robscheit-Robbins, F. S., and Whipple, G. H.: Blood Regeneration in Severe Anemias; Liver Fraction Potent in Pernicious Anemia Fed Alone and Combined With Whole Liver, Liver Ash, and Fresh Bile, J. Exper. Med. **49**: 215-227 (Feb.) 1929.
9. Furth, O., and Singer, K.: Liver Diet and Experimental Anemias, Zeitschr. ges. exp. Med. **69**: 126, 1929.
10. Whipple, G. H., and Robscheit-Robbins, F. S.: Simple Experimental Anemias and Liver Extracts, Proc. Soc. Exper. Biol. Med. **24**: 860, 1926-1927.
11. Robscheit-Robbins, F. S., Elden, C. A., Sperry, W. M., and Whipple, G. H.: Blood Regeneration in Severe Anemia: Influence of Inorganic Ash of Liver, Kidney, and Apricots, Proc. Soc. Exper. Biol. & Med. **25**: 416 (Mar.) 1928.
12. Whipple, G. H., Robscheit-Robbins, F. S., and Walden, G. B.: Blood Regeneration in Severe Anemia; A Liver Fraction Potent in Anemia Due to Hemorrhage, Am. J. M. Sc. **179**: 628, 1930.
13. Middleton, W. S.: The Erythropoietic Response of the Various Anemias to Liver Therapy, J. A. M. A. **91**: 855-863 (Sept.) 1928.
14. Dyke, S. C.: Liver Therapy in Secondary Anemia, Lancet **1**: 1192-1194 (June) 1929.
15. Powers, J. H., and Murphy, W. P.: Treatment of Secondary Anemia, J. A. M. A. **96**: 504-507 (Feb.) 1931.
16. Keefer, C. S., and Yang, C. S.: The Value of Liver and Iron in the Treatment of Secondary Anemias, J. A. M. A. **93**: 575-578 (Aug.) 1929.
17. Minot, G. R., Murphy, W. P., and Stetson, R. P.: The Response of the Reticulocytes to Liver Therapy: Particularly in Pernicious Anemia, Am. J. M. Sc. **175**: 581 (May) 1928.
18. Cohn, E. J., Minot, G. R., Fulton, J. F., Ulrichs, H. F., Sargent, F. C., Weare, J. H., and Murphy, W. P.: J. Biol. Chem. **74**: 69, 1927.
19. Robscheit-Robbins, F. S., and Whipple, G. H.: Am. J. Physiol. **83**: 76 (Dec.) 1927.
20. Hart, E. B., Steenbock, H., Waddel, J., and Elvehjem, C. A.: Iron in Nutrition: VII. Copper as a Supplement to Iron for Hemoglobin Building in the Rat, J. Biol. Chem. **77**: 797 (May) 1928.
21. Elvehjem, C. A.: The Relative Value of Inorganic and Organic Iron in Hemoglobin Formation, J. A. M. A. **98**: 1047-1050 (Mar.) 1932.
22. Lewis, M. S.: Iron and Copper in the Treatment of Anemia in Children, J. A. M. A. **22**: 175 (Feb.) 1930.
23. Josephs, H.: The Treatment of Anemia in Infancy, Am. J. Dis. Child. **39**: 1348 (June) 1930.
24. Mills, E. S.: Treatment of Idiopathic Anemia With Iron and Copper, Canad. M. A. J. **22**: 175 (Feb.) 1930.
25. Meyer, A. E.: Blood Regeneration in Dogs as Influenced by Iron Preparations. Paper Given at Division of Medical Chemistry, American Chemical Society, New Orleans, Mar. 28-31, 1932.
26. Starkenstein, H.: Arch. Exper. Path. Pharmacol. **127**: 101, 1928.
27. Reimann, F., and Fritsch, F.: Ztschr. klin. Med. **115**: 13, 1930.
28. Abderhalden, E.: Ztschr. f. Biol. **39**: 483, 1900.

LIVER EXTRACT IN THE TREATMENT OF DIABETES MELLITUS*

I. The Effect of Dried Liver Extract on Five Diabetic Children
II. The Effect of Dried Liver Extract on Adult Diabetic Patients

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INTRODUCTION

In 1927 and 1929 Murphy and Blotner[1, 2] reported the effect of whole liver on the blood sugar level of diabetic patients. A year later[3] they reported the effect of certain liver extracts on the blood sugar of such patients. This second paper contains an excellent review of the literature on the beneficial effect of liver extract in the treatment of diabetes, referring particularly to the work of Gilbert and Carnot,[4] Jousset,[5] Lamoreaux,[6] Gilbert and Lereboullet[7] and Lassance.[8] Experiments had been carried on by the French investigators[3, 4, 5, 6, 7, 8] using aqueous, alcoholic and saline extracts of liver in patients with diabetes mellitus. The conclusions were much alike. Apparently the oral administration of liver extracts had an appreciable influence on the glycosuria in a number of diabetic patients, the effect varying according to the case. During liver therapy the glycosuria disappeared or was diminished in some cases, whereas in others it was increased. The effects of the substance continued for a period of time after the treatment ceased. They felt that the cases which were favorably influenced by liver extract therapy were associated with a functional insufficiency of the liver while the cases which did not show improvement or had been made worse were those in whom the glycosuria depended on a hyperactivity of the liver. Lamoreaux[6] suggested that liver either acted by increasing the accumulation of glycogen in the liver or that acting on the whole organism it resulted in a more rapid destruction of sugar.

Blotner and Murphy[3] treated four diabetic patients with liver extracts over varying periods of time. The determinations were made for twenty-nine days on one case, thirty-one days on the second, sixty-eight days on the third case and five months on the fourth case. In the patient receiving the liver extract for twenty-nine days, the blood sugar level was lower than previously, and when seven units of insulin were injected twice daily, the blood sugar level was slightly lower than during the period of liver extract therapy alone. In the second case, the blood sugar level during liver extract therapy was at a slightly higher level than during the period when thirteen units of insulin was taken. In the third case the blood sugar remained at a lower level when liver extract was taken and

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increased when it was omitted. In the fourth case the blood sugar was stabilized by the liver extract and the urinary sugar was decreased. In their summary they suggest that liver contains a blood sugar reducing substance active when taken by mouth.

In studying the effect of any drug other than insulin on a diabetic patient the observations should be carried on over a considerable period of time. There are well recognized reasons for this. A properly treated diabetic on diet alone will gradually regain a certain amount of his carbohydrate tolerance, and if he adheres strictly to diet he may remain sugar free with a relatively low blood sugar level for an indefinite length of time. In the same way certain diabetic patients treated with insulin and diet may omit insulin for varying periods of time, sometimes several months, and will remain sugar free, their tolerance having been greatly increased during the period of insulin therapy. Obviously one cannot attribute the fact that the urine remains sugar free to the drug one is endeavoring to substitute for insulin until the patient has been observed for a period of several months.

In addition to the direct effect of liver and liver extracts on the blood sugar level, there has been a considerable amount of experimental work on the effect of liver injury on carbohydrate metabolism. Ravdin[9] found that the liver of jaundiced dogs kept on a mixed diet containing considerable amounts of carbohydrate contained only 1.4 per cent glycogen per 100 gm. of liver, whereas the liver of normal dogs on the same diet contained an average of 5.5 per cent glycogen. The blood sugar curves in these animals were distinctly elevated after the production of jaundice. He felt that his results indicated that the liver of the jaundiced animal was unable to store glycogen to the same extent as that of the normal dog. His work confirmed the finding of various other investigators, including Hetenyi,[10] who showed that in patients with liver disease, the blood sugar rise after 100 gm. of dextrose was higher and more prolonged than in normal people. Ferguson[11] found the same true in dogs after experimental ligation of the common duct and cholecystectomy. Observations of this kind indicate that a deficiency of the glycogen forming function of the liver may result from injury or disease of that organ. It is possible that such a deficiency may occur before there is any clinical or laboratory evidence of liver dysfunction. Forsgreen,[12] in normal rabbits, found that the percentage of glycogen and bile varied inversely to each other; the bile being greatest in amount when the glycogen was least. The latter was first deposited around the central veins of the lobule and remained there longest. One might infer, from these observations, that the primary disturbance in glycogen storage follows some pathologic change in the region of the central veins.

Chloroform and ether anesthesia, which markedly injure the liver, give rise to a pronounced hyperglycemia. Rosenthal and Bourne[13] found a definite interference with hepatic function after such anesthesia. Davis, Hall and Whipple[14, 15] and also Ravdin[9] found that liver cells regenerate rapidly on a high carbohydrate diet. This makes one realize again the importance of diet to the diabetic individual and suggests that the loss of glycogen from the liver in diabetic patients is in itself a cause of liver damage.

TABLE I

COMPARISON OF FERMENTABLE AND NONFERMENTABLE BLOOD SUGARS IN PATIENTS TREATED WITH LIVER EXTRACT, INSULIN AND LIVER EXTRACT, DIET ALONE AND INSULIN

LIVER EXTRACT			INSULIN AND LIVER EXTRACT			DIET ALONE			INSULIN		
TOTAL BLOOD SUGAR MG.	NONFERM. BLOOD SUGAR MG.	FERM. BLOOD SUGAR MG.	TOTAL BLOOD SUGAR MG.	NONFERM. BLOOD SUGAR MG.	FERM. BLOOD SUGAR MG.	TOTAL BLOOD SUGAR MG.	NONFERM. BLOOD SUGAR MG.	FERM. BLOOD SUGAR MG.	TOTAL BLOOD SUGAR MG.	NONFERM. BLOOD SUGAR MG.	FERM. BLOOD SUGAR MG.
107.1	16.6	90.5	97.8	13.1	84.7	80.0	13.8	66.2	52.3	11.1	41.2
110.3	18.0	92.3	104.1	11.9	92.2	81.0	13.5	67.5	73.2	11.1	62.1
120.0	12.0	108.0	136.3	15.6	120.7	83.0	14.3	68.7	83.3	20.8	62.5
121.0	12.1	108.9	148.5	12.5	136.0	87.0	14.7	62.3	95.2	14.7	80.5
125.0	11.1	113.9	156.2	14.7	141.5	90.0	18.9	71.1	105.0	15.6	89.4
128.1	12.8	115.3	166.6	10.4	156.2	105.0	12.5	92.5	108.7	13.1	95.6
128.1	13.1	115.0	176.4	11.9	164.5	114.0	13.8	100.2	116.2	10.6	105.6
130.3	13.8	116.5	187.5	9.6	177.9	131.5	14.1	117.4	117.1	12.0	105.1
133.9	11.0	122.9	194.8	11.1	183.7	137.0	12.5	124.5	129.0	15.6	113.4
141.4	10.0	131.4	194.8	15.1	179.7	142.0	15.6	126.4	130.3	11.9	118.4
141.4	12.5	128.9	202.7	10.8	191.9	153.0	14.2	138.1	135.1	10.6	124.5
141.4	9.6	131.8	211.2	22.7	188.5	166.0	13.3	152.7	136.3	10.2	126.1
141.4	12.1	129.3	211.2	15.6	195.6	166.0	15.4	150.6	137.6	13.8	123.8
145.7	11.9	133.8	234.5	11.3	223.2	210.0	10.0	200.0	141.4	16.0	125.4
150.0	12.5	137.5	250.0	12.5	237.5	250.0	12.0	238.0	142.8	10.0	132.8
156.2	9.6	146.6	250.0	12.5	237.5	285.0	13.2	271.8	150.0	12.5	137.5
163.0	12.6	150.4	263.1	12.1	251.0				157.8	11.7	146.1
164.8	12.1	152.7	312.5	10.0	302.5				166.7	15.1	151.6
166.6	11.3	155.3	312.5	13.8	298.7				168.5	29.4	139.1
176.4	10.8	165.6							169.0	27.7	141.3
200.0	13.2	186.8							187.5	28.4	159.1
200.0	11.9	188.1							200.0	14.7	185.3
									210.0	12.7	197.3
									219.0	13.1	205.9
									235.0	15.4	219.6
									235.0	14.3	220.7
									243.9	16.6	227.3
									246.1	15.3	230.8
									263.1	31.0	232.1
									272.7	14.7	258.0
									277.0	13.2	263.8
									294.1	15.1	279.0
									300.0	12.5	287.5
									300.0	13.1	286.9
									307.0	13.8	293.2
									312.4	16.6	295.8
									319.1	11.3	307.8
									326.0	12.5	313.5
									333.3	10.4	322.9
									378.3	29.4	348.9
									394.7	11.9	382.8
									444.4	14.5	429.9

PROCEDURE

The effect of the powdered liver extract has been observed in young and adult diabetic patients. The extract was made by the Lederle Laboratories using a slight modification of the procedure described by Blotner and Murphy.[3] In our observations we used mainly the dried extract as the original observers found this as effective as the moist paste and it was much easier for the patient to take. The amounts of extract used in each case are reported in the equivalents of the amount of raw liver from which the extract was derived. Usually 400 gm. of raw liver yielded about 2.5 gm. of the dried extract. The patients observed received the equivalent of from 400 to 1600 gm. of raw liver. In some cases the dose was increased to the equivalent of 2000 gm. The liver extract was given in divided doses one hour before meals. In some instances a dose was given on retiring at night. The patients had been treated in the diabetic clinics of the University and Bellevue Hospital Medical College, New York University, and the Third (New York University) Medical Division, Bellevue Hospital, for periods varying from two years to two months prior to treatment with liver. The young diabetics ranged in age from ten to nineteen years. The adult group included patients from thirty-four to sixty-one years of age. It has been suggested by several investigators that there are two types of diabetes, one in which the predominant disturbance is that of the oxidation of carbohydrate, the other in which the predominant disturbance is one of storage. It was felt that these two groups *might* present the two types of disturbance, provided one agrees that these two types exist and are sufficiently differentiated. The younger group, which consists invariably of the very severe diabetic, would be the type where the predominating disturbance was oxidative.

The relation of the fermentable and nonfermentable blood sugars in patients on insulin, on insulin and liver, on liver alone and on diet alone was also studied.

Liver function tests were done on all but one of the patients. These are reported in Table II.

Both fasting and after breakfast blood sugar determinations were made. The latter are indicated in the charts by a No. 2 at the level of the blood sugar reading. All blood sugar determinations were done by the Folin-Wu[16] method. The cholesterol determinations were done by the modified Bloor[17] technic.

RELATION OF FERMENTABLE TO NONFERMENTABLE SUGARS IN THE BLOOD IN PATIENTS TREATED WITH DIET, INSULIN, INSULIN AND LIVER EXTRACT, AND LIVER EXTRACT ALONE

Table I shows that there is no significant alteration in the relation of the fermentable to the nonfermentable sugars of the blood in patients treated with and without insulin, or with liver extracts. The highest nonfermentable blood sugars were found in the group treated with insulin alone but the average of the group was only 2 and 3 mg. higher than the other three groups. Apparently liver extract did not alter the ratio of fermentable and nonfermentable blood sugars.

RESULTS OF THE LIVER FUNCTION TESTS

All of the usual liver function tests were performed. In addition, on Cases 5, 6, 8, and 9 the recently reported test of Harrop and Baron,[18] in which bilirubin is injected intravenously, was used. The patients who showed some interference with liver function (Cases 5 and 8 with bromsulphalein and Cases 5, 6, and 8 with the injected bilirubin) were not patients who showed any definite improvement as a result of liver extract treatment. The liver function tests were done by Dr. Norman Joliffe.

THE EFFECT OF LIVER EXTRACT ON YOUNG DIABETICS

Five young diabetics were treated with liver extract. The cases were:

Case 1C, female, aged 15 yr., M.S.
2C, female, aged 15 yr., R.C.
3C, male, aged 15 yr., A.B.
4C, male, aged 10 yr., V.K.
5C, male, aged 15 yr., B.S.

The cases are not reported in detail because of lack of space. All blood sugars on the children were taken two hours after breakfast. Tables II, III, IV, V, and VI show the blood sugar levels.

TABLE II
LIVER FUNCTION TESTS

CASE	UROBILINOGEN URINE	BROMSULPHALEIN	INJECTED BILIRUBIN	VAN DEN BERGH			ICTERUS INDEX MG. PER CENT
				DIRECT	DELAYED	INDIRECT	
1	Normal	Less than 5 per cent					2.19
2	Normal	Negative		−		+	0.4
3	Normal	5 per cent			+		0.87
4	Normal	Faint Trace					
5	Normal	Negative	4th hr. 23.7%	−		+	0.5
6	Normal	Negative	4th hr. 59%	−		+	0.3
8	Normal	20 per cent	3rd hr. 60%	−		+	0.5
9	Normal	Negative	4th hr. Neg.	−		+	0.6
11	Normal	Negative		−		+	1.0
14	Normal	Negative		−		+	0.7
18	Normal	Negative		−		+	0.6

SUMMARY OF CASE 1.—Admitted to the diabetic clinic in August, 1929, at which time the child weighed 69 pounds. She required from 60 to 75 units of insulin to remain sugar free during 1929, the diet during this year being carbohydrate, 115 gm., protein, 50 gm., fat, 102 gm. The weight increased to 85 pounds during this time. During the year 1930 the carbohydrate in her diet

TABLE III

CASE 1C.—M. S.

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.
153.0		429.4	242.4	217.4	229.8
200.0	385.0	400.0	267.0	357.1	229.8
322.5		375.0	236.1	416.6	
384.0	230.1	428.5	202.0		
		288.4	256.4		
		300.0	233.9		
		306.1	272.1		
		500.0	238.0		
		428.5	242.4		
		326.0	233.9		
		405.4			
		385.0	246.9		
		340.9	248.7		
		272.7	253.7		
		500.0	261.5		

was increased to 150 gm., the fat averaged from 90 to 115 gm. The insulin requirement during this year varied from 60 to 70 units. In August of this year, the patient was admitted to the hospital in diabetic coma. Up to September, 1930, the weight of this patient had increased to 99 pounds. On September 17, 1930, the patient was receiving 57 units of insulin on a diet of carbohydrates, 150 gm., proteins, 65 gm., fats, 115 gm. and dried liver equivalent to 400 gm. was started. The blood sugars during the period of liver therapy are reported in Table II. The insulin was reduced to 45 units on September 24 and liver extract was increased to an amount equivalent to 1200 gm. of raw liver. The patient continued on 45 units of insulin without any appreciable effect on the blood sugar level and continued to spill sugar in varying amounts in after-breakfast specimens. On September 17, the patient showed acetone. On September 19, patient was taken off liver owing to the presence of acetone and large amounts of sugar in the urine and the insulin was increased to 60 units. At the time of this report this patient required 70 units of insulin daily. The dried liver extract had no effect on this patient's blood sugar level or on the glycosuria.

CASE 2C.—R.C. was admitted to the diabetic clinic March 26, 1929. Her weight was 90 pounds, blood sugar 268 mg., diet carbohydrates, 111 gm., proteins, 55 gm., fats, 105 gm., and insulin 60 units daily. During the year 1929, the child remained practically sugar free on this diet and required never less than 60 units a day. Her weight increased to 96 pounds. During 1930, the carbohydrate in the diet was raised to 136 gm. and patient was carried on from 40 to 60 units of insulin daily. On September 17, 1930, the patient was placed on the liver paste the equivalent of 400 gm. of raw liver and insulin was reduced from 40 to 25 units. The patient remained sugar free on the reduced insulin until October 2 at which time the after-breakfast specimen of urine showed 3 per cent sugar. Liver was increased to the equivalent of 600 gm. of the paste and the patient carried on 20 units of insulin, but the after-breakfast specimen con-

tinued to show sugar. There was no appreciable reduction in the blood sugar level during the treatment. The liver paste was increased on October 20 to the equivalent of 1000 gm. of raw liver. On November 3, dried liver extract equivalent to 1200 gm. was substituted for the wet liver extract. The patient's insulin at this time was 20 units a day. On November 15, the patient showed both sugar and acetone in the after-breakfast specimen. The insulin was raised to 30 units. As the patient continued *to spill sugar* and acetone after breakfast, the insulin was gradually raised to 70 units. Liver was discontinued on November 29 and on December 2 the patient was sugar free in the twenty-four hour specimen and the after-breakfast specimen. At the present time the amount of insulin has been reduced to 52 units and the patient is sugar free throughout the day. The blood sugar, after liver had been discontinued, showed a marked fall.

TABLE IV

CASE 2C.—R. C.

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.
268.0		241.9	278.2	197.3	317.4
262.0		272.7	251.6	120.0	266.6
333.0		131.5	261.5	110.3	220.0
250.0	263.0	256.4	261.5	57.3	229.8
230.8	256.4	238.2	242.4	187.5	226.0
333.3	256.4	205.4	206.4	157.8	208.3
		230.8	261.5	133.9	226.0
		205.4	238.0	88.2	
		300.0	242.4		
		340.9	226.7		
		277.7	187.7		
		250.0	187.7		
		357.1	250.7		
		326.0	242.4		
		300.0	253.5		

CASE 3C.—A.B., male. History of diabetes since 1922. Treated in this clinic since January, 1929, at which time the patient weighed 105 pounds, his blood sugar was 118 mg., and he was able to stay sugar free on 30 units of insulin daily and a diet of carbohydrates, 140 gm., proteins, 60 gm., fats, 120 gm. Blood sugars during 1929 went up on one occasion to 327 mg. but otherwise were never higher than 250 mg. Insulin requirement varied from 22 units to 65 units daily. Weight increased during this year to 114 pounds. During the year 1930, up to the time of liver therapy, the patient required from 65 to as little as 35 units to remain sugar free, depending on the amount of exercise. In September, 1930, the patient was taking 50 units of insulin and the carbohydrates in the diet had been raised to 185 gm. The patient was sugar free during the day but tended to show sugar after breakfast. On September 10, 1930, the patient was started on liver paste the equivalent of 1000 gm. of raw liver and 20 units of insulin were omitted, so that the patient was taking 30 units of insulin daily. The patient was sugar free for seven days except after breakfast. During this time, however, he lost four pounds. On the seventh day the twenty-four hour

specimen contained 5 gm. of sugar. The insulin was raised to 42 units. The patient continued to spill sugar for one week, the insulin was decreased to 40 units, then increased to 45 units. The patient on October 8 showed a concentration of 10 per cent sugar in his urine after breakfast, at which time the insulin was 45 units. The insulin was raised to 50 units and patient became sugar free. The insulin was cut to 45 units and the patient again spilled sugar. On October 21, at which time the patient was receiving 55 units of insulin, the after-breakfast specimen showed a 6 per cent sugar and 2-plus acetone with a blood sugar of 500 mg. Liver was withdrawn, insulin was raised to 70 units daily, and the patient became sugar free, but the blood sugar continued high. Liver was omitted until November 11, at which time the patient was placed on the dried liver extract, the equivalent of 800 gm. of raw liver. The amount of insulin at this time was 60 units. The after-breakfast specimen on November 15 showed both sugar and acetone. The patient was continued on this liver and insulin and was sugar free from November 18 to November 29 at which time the after-breakfast specimen showed 5 per cent sugar and 3-plus acetone. Liver was discontinued December 2. To render this patient sugar free it was necessary to raise the insulin to 82 units. This boy is extremely cooperative and has adhered strictly to his diet ever since he has attended our clinic. The diet at home was checked by the visiting dietitian. Liver obviously had no effect on the blood sugar of this young diabetic.

TABLE V

CASE 3C.—A. B.

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.
118.0		500.0	173.0	After First Period of Liver Extract	
222.0		375.0	233.9		
165.0		258.0	215.2		
166.0		394.7	190.5	428.6	177.7
200.0		150.0	224.9	576.9	196.0
251.0		375.0	185.0	468.6	199.0
327.0		163.0	202.0		
371.0	297.0	357.1	233.9	After the Second Period	
250.0	304.0	428.0	210.3		
285.0		157.8	177.7		
62.0	154.0	500.0	238.0	348.8	218.6
156.0				300.0	191.0
500.0		Second Period of Liver		400.0	173.0
				378.3	
				300.0	193.0
		441.0	222.2	250.0	166.6
		365.0	207.0	307.0	
		428.0	222.2	71.0	
		384.6	229.8	208.3	

CASE 4C.—V.K., aged ten, weight 79 pounds. Admitted to this clinic July, 1930. The patient was carried on a diet of carbohydrate, 140 gm., protein, 56 gm., and fat, 89 gm. Insulin requirement varied from 20 to 25 units. On September 10, the patient was placed on the equivalent of 400 gm. of dry liver

and the insulin was reduced to one dose of 10 units before breakfast. On September 13, the patient showed sugar in his after-breakfast specimen. The decreased dose of insulin was continued and the liver increased to the equivalent of 1200 gm. The patient was sugar free on September 20 but from then on showed sugar in his after-breakfast specimen. The total amount of insulin was increased to 16 units, then to 18, then to 20, then to 25. Liver was discontinued October 21. The patient required from 30 to 42 units of insulin to remain sugar free. The blood sugar level and glycosuria were not affected by the use of liver. At the time of this report this patient required 27 units of insulin daily and the carbohydrate in his diet had been raised to 165 gm.

TABLE VI

CASE 4C.—V. K.

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.
103.0		187.5	211.6	326.0	173.1
390.0		352.3	196.7	187.0	212.9
122.0	205.6	250.0	215.0	93.8	185.1
		272.7	211.6	172.4	211.6
		306.1	215.0	100.0	242.4
		182.9	222.2	91.5	222.7
		348.8	193.2	200.0	
		357.1	242.4	263.1	192.6
		441.0	193.2	220.5	168.7
		83.3	208.5	168.5	226.0
		327.8	187.7	394.7	
		319.1	192.4	159.6	277.7
				125.0	
				300.0	

CASE 5C.—B.S., aged sixteen. History of diabetes since 1926.

Treated in the diabetic clinic since 1929. The patient required an average of 65 units of insulin daily to remain sugar free on a diet of carbohydrates, 180 gm., proteins, 80 gm., and fats, 100 gm. The patient was hospitalized and the dried liver extract was started October 27, 1930, the equivalent of 1200 gm. of the raw liver. The patient required varying amounts of insulin during this period, the lowest being 59 units daily. On this amount, the patient was sugar free but the fasting blood sugar level was extremely high, never being less than 400 mg. This high blood sugar level was also noted in Case 3C. The patient remained sugar free, the amount of liver in the form of the dried extract being raised to the equivalent of 2000 gm. Insulin was cut to 69 units and on November 12, the patient showed a faint trace of sugar. On November 14, 25 gm. of sugar were spilled in the twenty-four hour period. The patient spilled again on November 17 and on November 20 and continued to spill from November 20 to December 5. The insulin during this time was raised to 75 units.

The patient was taken off liver on December 10 and was kept sugar free on 65 units of insulin daily. The blood sugar level remained high until December

29, at which time it was 75 mg. The blood sugar on January 2 was 83 mg. Insulin was then reduced to 50 units daily, then to 45 units and the patient was discharged from the hospital on 30 units of insulin daily. The present insulin requirement varies from 40 to 60 units daily.

TABLE VII

CASE 5C.—B. S.

BEFORE LIVER EXTRACT THERAPY		DURING LIVER EXTRACT AND INSULIN		INSULIN ALONE AFTER LIVER EXTRACT	
BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.	BLOOD SUGAR MG.	CHOLESTEROL MG.
300.0	242.4	267.8	242.4	535.7	196.3
272.7	272.1	500.0	266.6	428.6	215.7
		576.8	229.8		
		416.6	261.5	2nd Time	
		2nd Time on Liver		454.5	202.6
				428.5	193.2
				483.9	218.6
				454.5	192.6
		454.5	242.4	75.0	148.1
		454.6	203.9	83.3	
		500.0	202.0	52.3	222.2
		468.7	225.9	205.4	
		500.0	228.3	333.3	202.0
		500.0	261.5	164.8	182.6
		535.7	271.0	174.4	199.0
		576.9	222.2	87.2	177.7
		500.0	208.3	53.6	191.5
		384.6	186.7	441.0	199.9
		600.0	225.9	53.6	
		468.7	258.3		
		535.7	264.5		
		576.9	224.0		
		375.0			
		500.0	205.7		
		441.0	180.1		

SUMMARY OF THE CASES OF THE YOUNG DIABETICS TREATED WITH LIVER

Any one who has treated diabetic children realizes that the insulin requirement varies according to the child's activity and to whether or not any minor infection occurs. Four of these children were treated in the clinic and the insulin requirement was not materially altered during the period of liver therapy, nor was there any fall of the blood sugar level. On the contrary, three cases showed a definite tendency to a higher blood sugar level while on liver and insulin than when on insulin alone. One case was treated in the hospital. During the period of liver therapy no material change was effected in the amount of insulin, in fact less insulin was required to keep the patient sugar free after liver therapy was discontinued. The hospitalized case was one of the cases showing an elevated blood sugar level with no glycosuria during the period of liver therapy. In the study of these five cases, it seems justifiable to conclude that neither the dried nor the moist liver extract can replace or reduce the amount of insulin required by the young diabetic.

TABLE VIII
SUMMARY OF ADULT DIABETIC PATIENTS

CASE		AGE	SEX	ONSET OF DIABETES	PREVIOUS DIET				PRESENT DIET				TOTAL PERIOD ON LIVER (WKS.)	DRIED LIVER AMT. GIVEN IN EQUIVALENT OF GM. OF RAW LIVER
					INS.	C.	P.	F.	INS.	C.	P.	F.		
1	L. H.	47	F	1920	12	135	56	102	5	147	60	87	41	Av. 1800
2	T. W.	51	F	1926	35	150	64	150	15	199	63	98	59	400-1200 Av. 800
3	H. B.	42	F	1929	8	130	56	102	5	152	60	87	45	800-1600
4	A. G.	44	M	1925	8	142	78	136	5	142	78	111	45	800-1200
5	M. S.	61	M	1930	5	170	56	115	0	164	58	65	50	800
6	R. C.	46	M	1929	30	130	64	100	Not Known				23	1600
7	C. B.	34	F	1929	20	180	75	122	10	174	63	83	51	1200
8	R. K.	57	F	1930	15	130	56	90	10	130	56	90	40	800-2000
9	N. N.	39	M	1929	70	130	65	100	15	175	64	85	49	1600-2400
11	H. G.	55	F	1925	20	140	55	91	20	140	55	91	36	800-2000 Av. 1200
14	J. B.	52	F	1930	0	150	58	102	0	170	66	101	39	1200-1600
15	G. N.	53	F	1927	0	80	55	97	0	100	55	80	29	800
18	A. W.	35	F	1930	10	140	53	91	0	140	53	91	25	1200

THE EFFECT OF THE DRIED LIVER EXTRACT OVER A PROLONGED PERIOD OF TIME ON ADULT DIABETIC PATIENTS

The effect of the dried liver extract was observed on thirteen adult diabetic patients. The summary of these cases is shown in Table VIII. In several patients the moist and liquid extracts were used for short periods of time but were discontinued, as they were extremely unpleasant to take and as Blotner and Murphy's results did not show any difference in their effect from that of the dried extract. Cases 1, 6, and 9 were hospitalized for a period of time during the administration of the extract. Blood sugar, cholesterol, and urinary sugar determinations were made on all patients. The patients examined their urine by Benedict's qualitative method daily, and a twenty-four hour specimen of urine was examined each time the patient came to the clinic. In some cases, the patient returned weekly for examination, in others twice a week. The diet in each case was carefully checked. All of these patients weighed their food on scales and were instructed carefully about the necessity for accuracy and adhering to the diet as ordered.

The blood cholesterol determinations, done each time the patients reported for blood sugars, are not reported in detail owing to lack of space and also because there was no significant alteration as a result of liver therapy. The average blood cholesterols before and during liver therapy are reported in Table IX.

TABLE IX

AVERAGE OF THE BLOOD CHOLESTEROL DETERMINATION BEFORE AND DURING LIVER EXTRACT THERAPY ON THE ADULT PATIENTS

CASE	AVERAGE BLOOD CHOLESTEROL MG.	
	BEFORE LIVER EXTRACT TREATMENT	DURING LIVER EXTRACT TREATMENT
1	251.8	267.9
2	226.0	210.0
3	254.7	268.0
4	290.2	246.3
5	211.1	188.4
6	303.0	281.2
7	237.2	227.1
8	244.4	249.2
9	254.2	261.1
11	233.3	255.4
14	276.0	244.4
15	232.3	215.9
18	221.6	207.5

Of the 13 cases investigated all but 2 (Cases 14, 15) were on insulin prior to receiving liver extract. These 2 patients were on liver for a period of thirty-nine and twenty-nine weeks respectively, Case 14 receiving the equivalent of 1200 to 1600 gm. of raw liver and Case 15 receiving the equivalent of 800 gm. of raw liver. The charts on these patients show that there was no effect on the blood sugar level during the period of liver therapy (Charts 14 and 15).

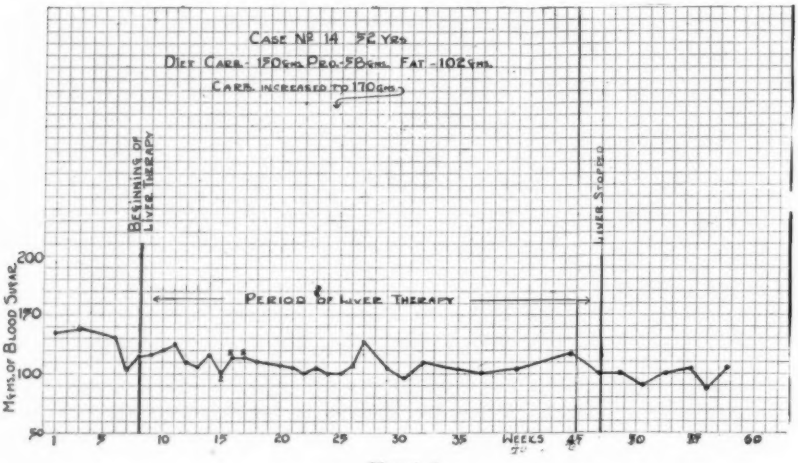

Chart 1.

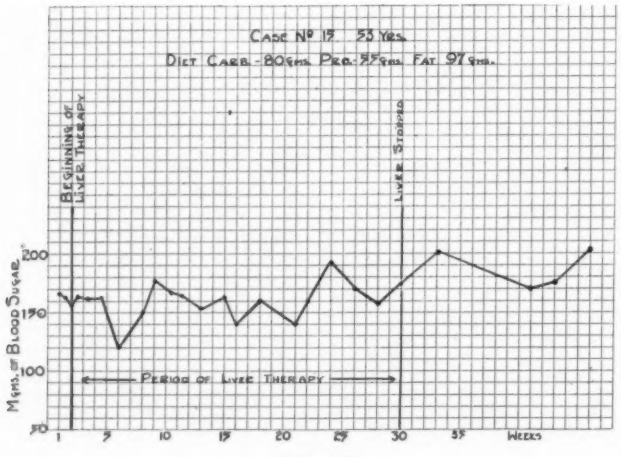

Chart 2.

Four patients (Cases 3, 4, 5, and 18) had required ten units of insulin or less daily. Following the use of liver extract, for periods of not less than twenty-five weeks and extending in 3 cases for forty-five weeks or more, there was no significant effect on the blood sugar level. It is true that all 4 patients were able to omit insulin for a period of time but in the treatment of diabetic patients who are on small doses of insulin this is a circumstance that often occurs and if the diet is adhered to strictly, insulin may be needed for only short periods of time. This is further brought out in all of these cases by the fact that when liver was

discontinued and the patient was treated without either insulin or liver, there was no striking increase in the blood sugar level (Charts 3, 4, 5, and 18).

The remaining 7 patients (Charts 1, 2, 6, 7, 8, 9, and 11) required 12 or more units of insulin daily and in this group, Cases 2 and 9 showed results which

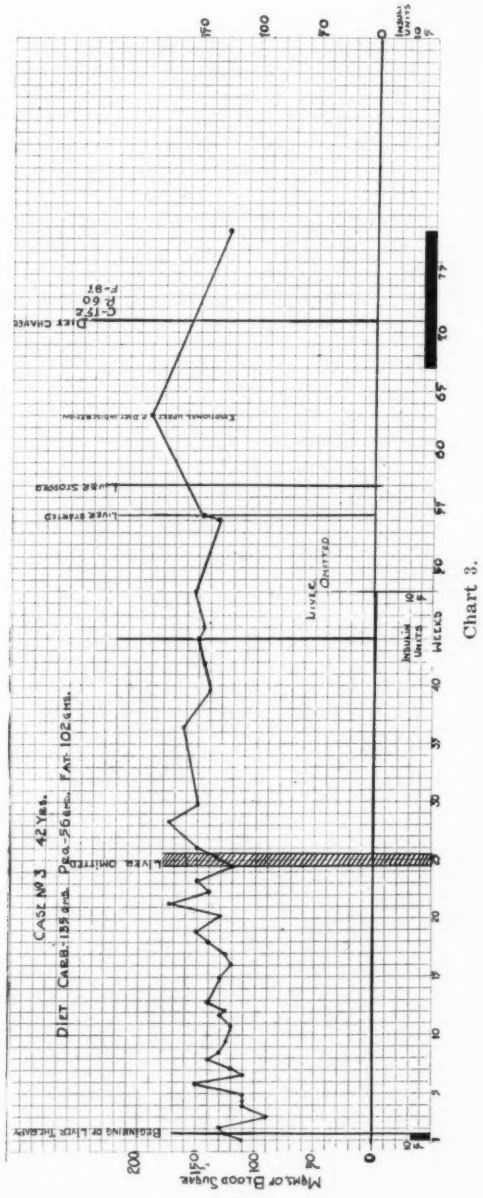

Chart 3.

might be attributed to the effect of the liver extract treatment. In the other 5 cases the reduction of the daily insulin dose was not greater than what might be expected as a result of continued adherence to diet. In addition, these patients continued with blood sugars of the same level following withdrawal of liver.

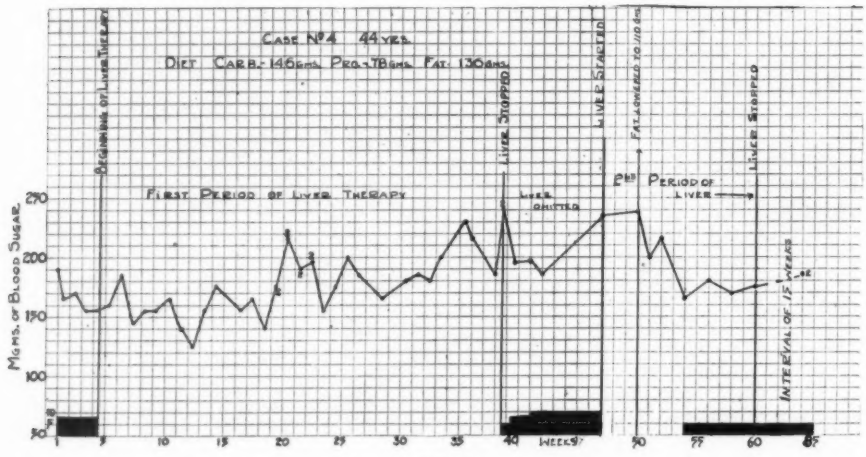

Chart 4.

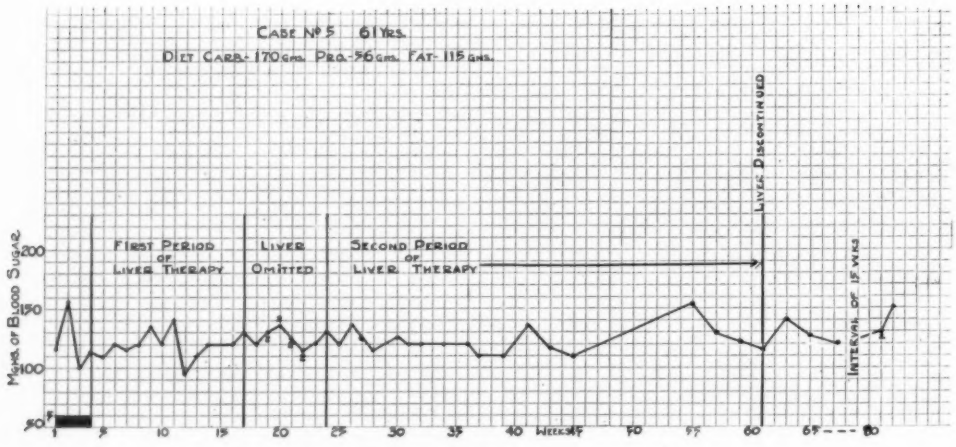

Chart 5.

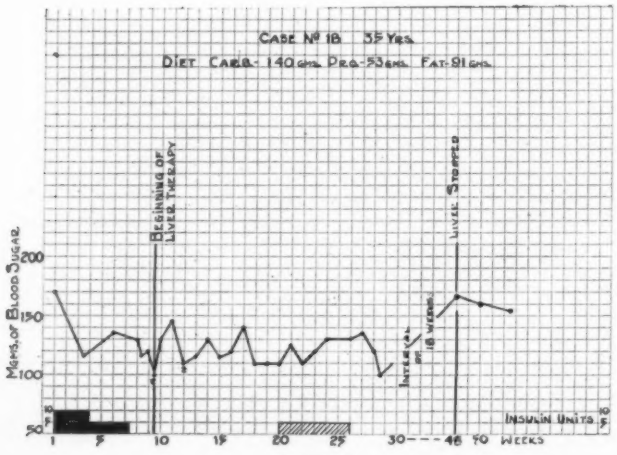

Chart 6.

Case 2 was able to reduce insulin dosage from 35 to 15 units daily. After this patient had been treated for a period of thirty-five weeks, the fat in the diet was reduced from 150 to 123 gm. and the carbohydrate increased from 150 to 171 gm. Following this change in diet there was a greater fall in the blood sugar level than had been encountered during any period of the treatment. This patient is at present tolerating a diet of:

Carbohydrate	200 grams
Protein	65 grams
Fat	100 grams

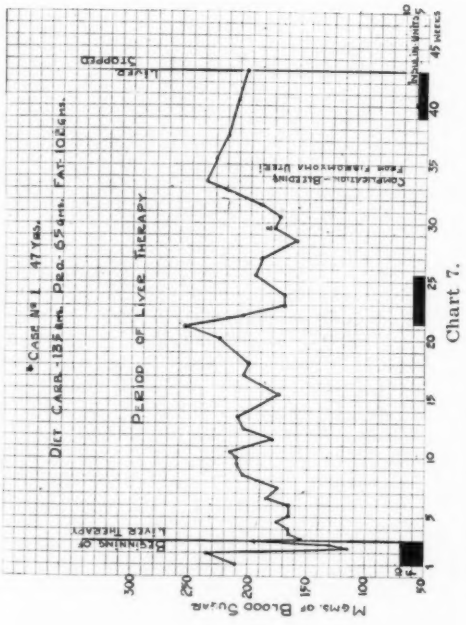
Case № 1 47 Yrs.
Diet Carb. 125 gm. Pro. 65 gm. Fat. 106 gm.
Period of Liver Therapy
Complication - Bleeding from Pharyngoma Uteri
Liver Stopped
Beginning of Liver Therapy
Mgms. of Blood Sugar
Weeks

Chart 7.

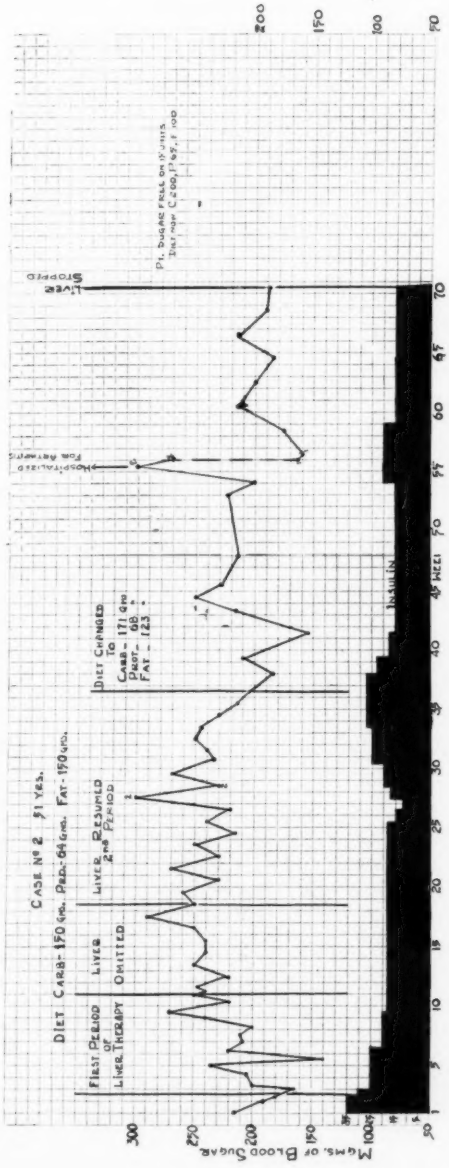
Case № 2 51 Yrs.
Diet Carb. 150 gm. Pro. 64 gm. Fat. 150 gm.
First Period of Liver Therapy
Liver Omitted
Liver Resumed 2nd Period
Diet Changed To Carb. - 171 gm Prot. - 68 " Fat - 123 "
Liver Stopped
Hospitalized for Arterio
Insulin
Mgms. of Blood Sugar
Weeks

Chart 8.

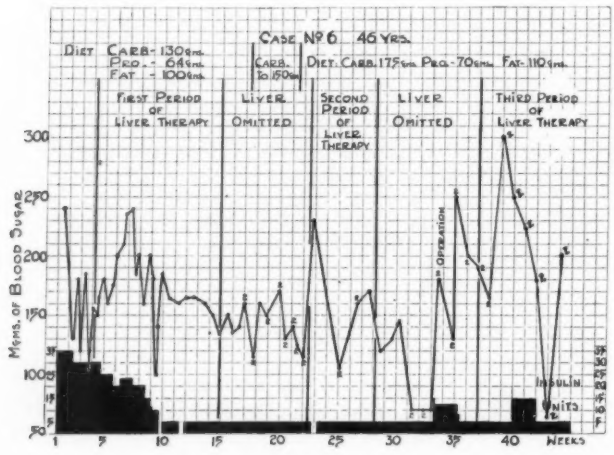

Chart 9.

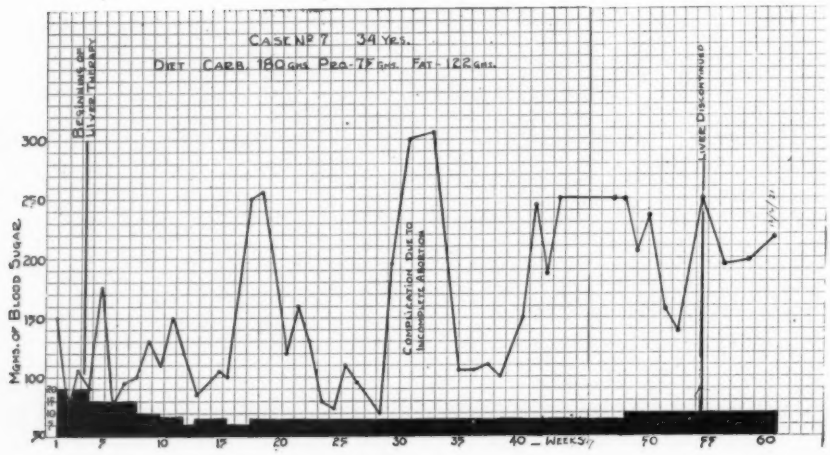
CASE Nº 7 34 YRS.
DIET CARB. 180 GMS. PRO.-75 GMS. FAT-122 GMS.
BEGINNING OF LIVER THERAPY
COMPLICATION DUE TO INCOMPLETE ABORTION
LIVER DISCONTINUED
MGMS. OF BLOOD SUGAR
WEEKS

Chart 10.

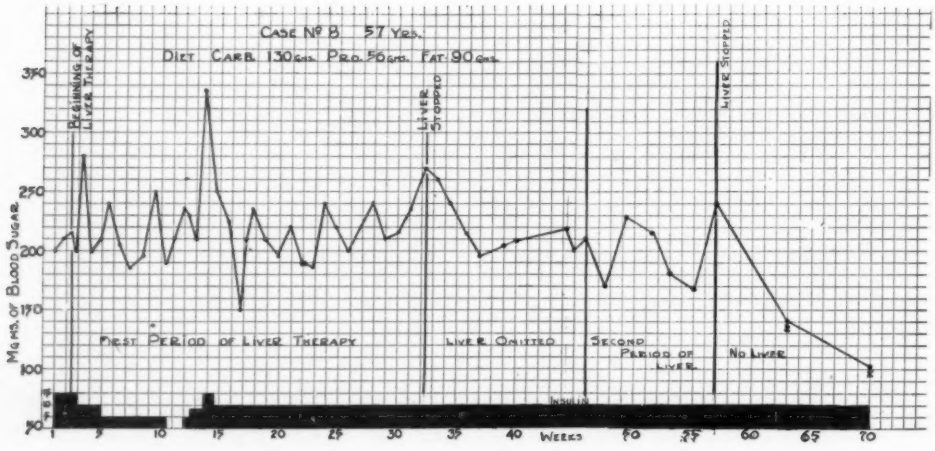

Chart 11.

and has remained sugar free on a daily dosage of 15 units of insulin. Case 9 is a patient who had been treated in our clinic since 1929 and had required an average of 70 units of insulin daily. During his first period of liver therapy, it was possible to reduce the insulin to 25 units daily. When liver extract was omitted, the blood sugar rose, and it was again necessary to increase the amount of insulin. During this period the patient was kept sugar free on 55 units of insulin, below which amount he invariably spilled sugar. During his second pe-

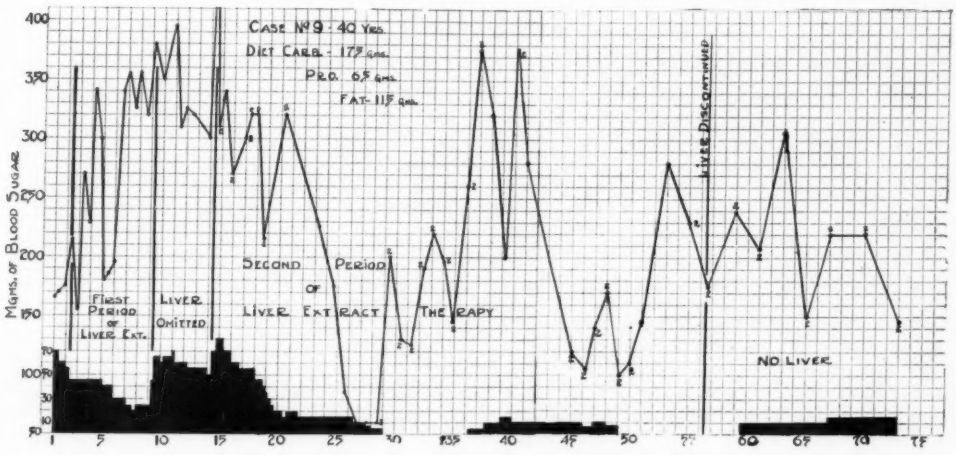

Chart 12.

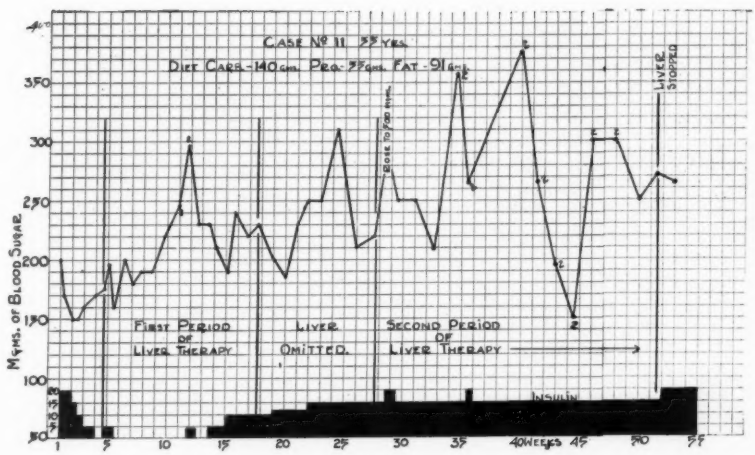
Case № 11 - 52 Yrs.
Diet Carb. - 140 Gms. Pro. 75 Gms. Fat - 91 Gms.
Liver Stopped
Mgms. of Blood Sugar
First Period of Liver Therapy
Liver Omitted
Second Period of Liver Therapy
Insulin
Weeks

Chart 13.

riod of liver therapy it was possible to reduce the insulin to 5 units daily and the blood sugar at this time was lower than at any time during the two years that the patient has been under observation. When insulin was withdrawn, the blood sugar immediately rose. Liver has been discontinued in this patient, and he has been on insulin alone for a period of over five months. His blood sugar level has remained below 200 mg. and he has been kept sugar free on a dose of 15 units daily.

SUMMARY AND DISCUSSION

Our results do not substantiate the findings of Blotner and Murphy.[1, 2, 3] In the young patients observed neither the dry nor the moist liver extract had any effect on the hyperglycemia, nor was it possible to reduce the insulin requirement during the period of liver therapy.

Of the adult patients only Case 9 seemed to show any marked improvement while receiving liver extract. In Case 2 it was possible to reduce the insulin but the hyperglycemia was affected more by the change in diet than it was by the liver therapy.

While these observations have been in progress other investigators have reported a similar lack of effect of liver extract in diabetes. Bowen and Sly[19] investigated the effect of whole liver and liver extract on the respiratory quotient of two diabetic patients and found no evidence that either had a favorable influence on the diabetes.

DePencier, Soskin and Best[20] studied the blood sugar and sugar excretion of depancreatized dogs after whole liver and were unable to observe any favorable results. Certain of the German investigators Habs,[21] Bertram, Horwitz and Wahneau[22] reported that neither liver nor liver extract have any appreciable influence on the blood sugar or glycosuria in diabetic patients.

In the treatment of diabetes one is always impressed with the ability of the patient to increase his carbohydrate tolerance by adherence to diet and insulin, and if these patients are followed over a period of time the results are, in the majority of cases, striking. This makes it difficult to draw any conclusions as to the effect of a given type of therapy on the course of the disease. In this instance the patients were observed for periods of not less than one year and received the extract for never less than five months and in most of the cases for more than five months. Therefore, it seems reasonable to conclude that if any improvement were to occur it would have taken place during this prolonged period of observation. In view of this fact one does not feel justified in attributing to liver extract any effect similar to that of insulin on the hyperglycemia or glycosuria of diabetic patients.

REFERENCES

1. Murphy, W. P., and Blotner, H.: The Effect of Liver Feeding on the Blood Sugar, Jour. Clin. Investigation **4**: 440, 1927.
2. Blotner, H., and Murphy, W. P.: The Effect of Liver on the Blood Sugar Level, J. A. M. A. **92**: 1332, 1929.
3. Blotner, H., and Murphy, W. P.: Effect of Certain Liver Extracts on the Blood Sugar of Diabetic Patients, J. A. M. A. **94**: 1811, 1930.
4. Gilbert, A., and Carnot, P.: Essais d'opotherapie hepatique, Semana méd. **16**: 480, 1896.
———, and ———: Note preliminaire sur l'opotherapie hepatique, Presse méd. No. 97, p. 422, 1896.
———, and ———: Action des extraits hepatique sur la glycosurie experimentale, Semana méd. **16**: 513, 1896.
———, and ———: De l'opotherapie hepatique dans le diabete sucre, Semana méd. **17**: 189, 1897.
———, and ———: Etudient l'opotherapie hepatique dans le diabete sucre, Gaz. d. hôp. No. 94, p. 995, 1900.
5. Jousset, M.: Opotherapie hepatique, Presse méd. No. 99, p. 432, 1896.
6. Lamoreaux, F.: De l'opotherapie hepatique dans le diabete sucre, Paris thesis, 659-98, 1897.
7. Gilbert, A., and Lereboullet, P.: Les opotherapies dans le diabete sucre, Gaz. hebd. de méd. No. 81, p. 963, 1901.
8. Lassance, V.: Les opotherapies dans le diabete sucre, Paris thesis, p. 394, 1905.

9. Ravdin, I. S.: Some Aspects of Carbohydrate Metabolism in Hepatic Disease, J. A. M. A. **93**: 1193, 1929.
10. Hetenyi, Geza: Deutsche med. Wchnschr. **48**: 420, 1922; **48**: 770, 1922; **48**: 1200, 1922.
11. Ferguson, L. K.: S. Clin. North America **8**: 861, 1928.
12. Forsgreen, E.: Skandin. Arch. f. Physiol. **53**: 137, 1928; **55**: 144, 1929.
13. Rosenthal, S. M., and Bourne, W.: Effect of Anesthetics on Hepatic Function, J. A. M. A. **90**: 377, 1928.
14. Davis, N. C., Hall, C. C., and Whipple, G. H.: Rapid Construction of Liver Cell Protein on Strict Carbohydrate Diet Contrasted With Fasting, Arch. Int. Med. **23**: 689, 1919.
15. Davis, N. C., and Whipple, G. H.: Liver Regeneration Following Chloroform Injury as Influenced by Various Diets, Arch. Int. Med. **23**: 711, 1919.
16. Folin, O., and Wu, H.: A Simplified and Improved Method for the Determination of Sugar, J. Biol. Chem. **41**: 367, 1920.
17. Bloor, W. R., Pelkan, K. F., and Allen, D. M.: J. Biol. Chem. **52**: 191, 1922.
18. Harrop, G. A., and Barron, E. S.: The Excretion of Intravenously Injected Bilirubin as a Test of Liver Function, J. Clin. Investigation **9**: 577, 1931.
19. Bowen, B. D., and Sly, G. E.: Effect of a Liver Extract on Blood Sugar and Respiratory Quotient in Diabetes, Proc. Soc. Exper. Biol. & Med. **29**: 593, 1932.
20. DePencier, M. T., Soskin, S., and Best, C. H.: Effect of Liver on Blood Sugar Level and on Sugar Excretion of Depancreatized Dogs, Am. J. Physiol. **94**: 548, 1930.
21. Habs, H.: Zur Frage einer Lebertherapie des Diabetes, Klin. Wchnschr. **10**: 935, 1931.
22. Bertram, Horwitz, and Wahneau: Experimentelle und klinische Untersuchungen zur Frage der peroralen Diabetes behandlung; Leberextrakte als Resorptionsvermittler des Insulins, Klin. Wchnschr. **10**: 1214, 1931.

THE EFFECT OF LIVER EXTRACT ON BILE PIGMENT FORMATION*

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THERE are a number of reports in the literature suggesting that liver extract may have some effect on bile pigment metabolism. Why an apparent retention of bilirubin occurs in pernicious anemia, as is demonstrated by the icteric index and lemon-yellow tinting of the tissues, is not known. That it is a definite feature of the disease is shown by the fact that when liver extract is given to the patient suffering from pernicious anemia, the icteric index decreases along with the reticulocyte response and increase in erythrocyte count. Murphy, Monroe and Fitz,[1] who first recorded this fact, suggested that the disappearance of bilirubin from the blood and tissues might mean that it was being utilized in the formation of hemoglobin by the bone marrow in response to the liver treatment. The other possibilities are that the liver extract might increase the elimination of bile pigment in the bile and that it may prevent excessive formation of bile pigment. Verzar[2] has suggested that bilirubin is the "hematopoietic hormone" and being a by-product of red cell destruction, regulates their formation and liberation into the blood stream. He reports that in rabbits, bilirubin in small doses stimulates erythropoiesis and in large doses inhibits, and that in anemic rabbits, regeneration of erythrocytes occurs more rapidly if bilirubin is administered. It remains to be proved whether this concept is true; however, it is related theoretically to the apparent retention of bilirubin in pernicious anemia. Jungmann[3] and Schulten[4] hold that liver therapy inhibits the hemolytic apparatus, since all signs of increased blood destruction are diminished. On the other hand, Singer[5] has shown that the daily stercobilin output in the normal

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individual is not decreased on liver therapy, and Harrop and Barron[6] have found that the ability of the liver of the pernicious anemia patient to eliminate bilirubin injected intravenously is less than normal. Further, it is known that jaundiced and biliary fistula dogs frequently develop an anemia, and Whipple and Hooper[7] have reported the biliary fistula dogs improve nutritionally when fed cooked liver. The constituent of liver that is responsible for this improvement is not known. It occurred to us, therefore, that the effect of liver extract on bile pigment output should be studied and that it should be ascertained if liver extract contained the principle responsible for the improvement of biliary fistula dogs.

METHODS

For this work biliary fistula dogs were prepared according to the method of Rous and McMaster.[8] The dogs were kept in cages and given no exercise because of the known influence of exercise on bile pigment formation.[9] The dogs were fed a stock diet of cooked yellow corn meal and bread mixed with bone broth. We used such a carbohydrate diet because the bile pigment elimination on such a diet is low[10] and we thought significant changes would be more readily detected. The dogs were kept on the diet for one week before the operation and thereafter. During the test period the dogs were given the extract of 200 gm. of liver (Eli Lilly, No. 343) daily. The bile was collected from the balloon twice daily and the bile pigment estimated immediately. Thannhausen and Andersen's modification of the Van den Bergh method for serum bilirubin[11] was used. This method was compared with that of Rous and McMaster[12] and the results checked. The liver extract was found to contain no bile pigment and only a trace of bile salts.

RESULTS

Thirteen dogs were prepared with a biliary fistula. Eight lived from twenty-two to forty days; the other two died at twenty days from peritonitis due to a perforated duodenal ulcer. Three dogs were fed daily with "gastric mucin" (Armour) and lived indefinitely in excellent condition.

All but three of the animals were in good condition during the period that data were collected. From one week to ten days after the operation, the bile and pigment output became uniform.[10]

Typical results are shown in Tables I and II. These were obtained on Dogs 5, 8, 10, and 11. A fairly uniform excretion of bile pigment occurred during the control and liver extract feeding periods, but occasionally an inexplicable increase in the twenty-four hour output of bile occurred.

The twenty-four hour output of bile on the diet fed varied from 5 to 15 c.c. per kilo body weight. The range of bile pigment output varied from 1 to 3 mg. per kilo body weight in twenty-four hours.

The liver extract administration did not influence bile or bile pigment output in these dogs. Neither could it be said that the liver extract improved the nutritional condition of the dogs, but Fogelson's "gastric mucin" did. Duodenal or gastric ulcer has not appeared in another series of seventeen biliary fistula dogs on gastric mucin.[13]

TABLE I

SHOWING NO EFFECT OF LIVER EXTRACT ON BILE PIGMENT OUTPUT IN DOGS 5 AND 8

OPERATION DAYS AFTER	AMT. C.C. BILE IN 24 HR.		AMT. IN MG. BILE PIGMENT IN 24 HR.	
	TOTAL	PER KILO BODY WT.	TOTAL	PER KILO BODY WT.
		Dog 5—Male		
		Stock diet		
8	150	9.7	22.5	1.5
9	140	9.0	25.6	1.7
10	155	10.3	21.9	1.5
Average	148	9.7	23.3	1.6
		Stock diet with liver extract		
11	170	11.3	27.1	1.8
12	170	11.3	41.7	2.3
13	195	13.4	41.8	2.9
14	185	12.7	37.5	2.6
15	150	10.0	27.7	1.9
16	189	14.0	31.7	2.3
17	175	12.5	26.3	1.9
Average	178	12.2	33.0	2.3
		Stock diet		
18	200	14.7	37.5	2.8
19	185	13.6	29.6	2.2
20	88	6.7	4.8	0.3
21	235	20.5	30.9	2.3
22	226	18.9	25.3	1.9
23	220	17.7	36.2	2.8
24	210	16.7	32.3	2.6
Average	195	13.8	28.1	2.1
		Dog 8—Male		
		Stock diet with liver extract		
8	125	8.2	22.9	1.5
10	125	9.0	26.3	1.8
12	140	10.0	40.4	2.9
14	100	7.5	34.9	2.6
Average	123.2	8.7	31.1	2.2
		Stock diet		
9	140	9.6	27.6	1.9
11	140	9.8	43.6	3.0
12	130	9.4	33.6	2.4
15	98	7.5	34.2	2.6
Average	127	9.1	35.0	2.5

DISCUSSION

The data obtained show that liver extract does not influence the formation of bile pigment or bile output in biliary fistula animals. However, from this data it does not necessarily follow that liver extract might not have a favorable action on bile pigment elimination and metabolism in patients with pernicious anemia. This is substantiated by the work of Singer,[5] who found that liver therapy did not influence the stercobilin output in the feces of normal subjects, which checks with our results, and by the recent observations of Paschkis and Diamant[15] who found a decrease in stercobilin output in pernicious anemia patients on the institution of liver therapy, the decrease in stercobilin output preceding or accompanying the improvement in the blood picture. Whether this decrease in bile pigment output in pernicious anemia patients is due to decreased blood destruction, or to the utilization of bilirubin in hemoglobin manufacture, or to some qualitative change in erythropoiesis is yet to be determined.

TABLE II

SHOWING NO EFFECT OF LIVER EXTRACT ON BILE PIGMENT OUTPUT IN DOGS 10 AND 11

OPERATION DAYS AFTER	AMT. C.C. BILE IN 24 HR.		AMT. IN MG. BILE PIGMENT IN 24 HR.	
	TOTAL	PER KILO BODY WT.	TOTAL	PER KILO BODY WT.
		Dog 10—Male		
		Stock diet with gastric mucin, 30 gm. per day		
10	85	11.6	21.2	2.9
11	80	10.0	18.7	2.4
12	105	13.4	18.7	2.3
13	95	12.3	17.8	2.3
14	90	11.7	19.2	2.4
Average	91	11.8	19.1	2.5
		Stock diet with gastric mucin and liver extract		
15	100	13.1	17.8	2.3
16	90	11.7	19.5	2.5
17	100	13.1	22.0	2.9
18	95	13.5	16.1	2.3
19	105	14.6	19.2	2.6
Average	98	13.2	18.9	2.5
		Dog 11—Female		
		Stock diet with gastric mucin		
17	87	9.5	19.7	2.1
18	95	11.3	21.5	2.5
19	90	10.0	19.8	2.2
20	90	10.2	21.0	2.3
Average	90.5	10.3	20.5	2.5
		Stock diet with gastric mucin and liver extract		
21	95	11.0	26.1	3.0
22	102	11.5	23.9	2.9
23	120	13.8	18.2	2.0
24	125	14.7	17.3	2.0
Average	110.5	12.7	21.4	2.5
		Stock diet with gastric mucin		
27	135	15.3	19.3	2.1
26	120	13.8	21.8	2.6
27	135	15.3	19.3	2.1
28	135	15.5	24.9	2.8
Average	129	14.7	21.1	2.4

The range of bile excreted in twenty-four hours in our dogs was approximately the same as that found by McMaster and Rous,[10] who observed a lower range of from 1 to 7 c.c. and an upper range of from 8 to 14 c.c. per kilo. Our results on bile pigment output are lower than those of Hooper and Whipple[7] (9 mg. per kilo) and those of Rous and McMaster[8, 10] (6 to 7 mg. per kilo). Our low figure is due to the diet, since the figures of the workers just referred to were obtained on diets containing meat and liver.

Duodenal ulcers were found in three dogs in this series of ten without mucin, perforation of the ulcer being the cause of death in two.[13] The occurrence of duodenal ulcers in biliary fistula dogs has been reported by most workers and more recently by Berg and Jobling.[14] How gastric mucin operates to prevent ulcer formation is not known.[13]

CONCLUSION

Liver extract (the fraction active in pernicious anemia) given by mouth does not influence the output of bile or bile pigment in biliary fistula dogs. Neither does it contain the active constituent of liver which improves the nu-

tritional condition in biliary fistula dogs. "Gastric mucin" maintains biliary fistula dogs in excellent nutritional condition.

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REFERENCES

1. Murphy, W. P., Monroe, R. T., and Fitz, R.: Changes in Composition of Blood in Pernicious Anemia Treated by Diet Rich in Liver, J. A. M. A. **88**: 1211, 1927.
2. Verzar, F.: Die Rolle des Bilirubins bei der Regulation der roten Blutkörperchenzahl, Ztschr. f. d. ges. exper. Med. **68**: 475, 1929.
3. Jungmann, P.: Über die Wirkungsweise der Leberdiät bei der perniziösen Anämie, Klin. Wchnschr. **7**: 441, 1928.
4. Schulten, H.: Zur Lebertherapie der Perniziösen Anämie. Fortlaufends Beobachtung von 25 leberbehandelten Pernizio-sakranken, München. med. Wchnschr. **76**: 1281, 1929.
———: Der gegenwärtige Stand der Lebertherapie der Perniziösen Anämie, Klin. Wchnschr. **8**: 1820, 1929.
5. Singer, K.: Studien zum Problem der Blutmauserung; über den Einfluss der Ernährung auf die Urobilinogenausscheidung mit den Fäzes, Wien. Arch. f. inn. Med. **20**: 59, 1930.
———: Studien zum Problem der Blutmauserung; über den Einfluss der Leberdiät auf die Funktion des Erythrolytischen Systems, Ztschr. f. d. ges. exper. Med. **71**: 137, 1930.
6. Harrop, G. A., Jr., and Barron, E. S. G.: Excretion of Intravenously Injected Bilirubin as Test of Liver Function, J. Clin. Investigation **9**: 577, 1931.
7. Whipple, G. H., and Hooper, C. W.: Bile Pigment Metabolism, II. Bile Pigment Output Influenced by Diet, Am. J. Physiol. **40**: 349, 1916.
Whipple, G. H., and Hooper, C. W.: Bile Pigment Metabolism, III. Bile Pigment Output and Blood Feeding, Am. J. Physiol. **42**: 256, 1917.
Hooper, C. W., and Whipple, G. H.: IV. Influence of Fresh Bile Feeding Upon Whole Bile and Bile Pigment Secretion, Am. J. Physiol. **42**: 264, 1917.
Hooper, C. W., and Whipple, G. H.: The Influence of Bile Constituents on Bile Pigment Secretion, Taurocholic, Glycocholic and Cholic Acid, Am. J. Physiol. **42**: 280, 1917.
8. Rous, P., and McMaster, P. D.: Method for Permanent Sterile Drainage of Intraabdominal Ducts as Applied to Common Duct, J. Exper. Med. **37**: 11, 1923.
9. Brown, G. O.: Blood Destruction During Exercise; Blood Changes Occurring in Course of Single Day of Exercise, J. Exper. Med. **36**: 481, 1922.
———: Blood Destruction During Exercise; Demonstration of Blood Destruction in Animals Exercised After Long Confinement, J. Exper. Med. **37**: 113, 1923.
———: Blood Destruction During Exercise; Exercise as Bone Marrow Stimulus, J. Exper. Med. **37**: 187, 1923.
———: Blood Destruction During Exercise; Development of Equilibrium Between Blood Destruction and Regeneration After a Period of Training, J. Exper. Med. **37**: 207, 1923.
10. Rous, P., Brown, G. O., and McMaster, P. D.: Studies on Total Bile; Effects of Operation, Exercise, Hot Weather, Relief of Obstruction, Intercurrent Disease, and Other Normal and Pathological Influences, J. Exper. Med. **37**: 395, 1923.
11. Thannhauser, S. J., and Anderson, E.: Bilirubin in Blood Serum, Deutsches Arch. f. klin. Med. **137**: 179, 1921.
12. Rous, P., and McMaster, P. D.: Concentrating Activity of Gall Bladder, J. Exper. Med. **34**: 47, 1921.
13. Kim, M. S., and Ivy, A. C.: J. A. M. A. (In press.)
14. Berg, B. N., and Jobling, J. W.: Biliary and Hepatic Factors in Peptic Ulcers, Experimental Study, Arch. Surg. **20**: 997, 1930.
15. Paschkis, K., and Diamant, M.: Beitrage zur Pathologie der perniziösen Anämie. Zugleich II. Mitteilung über den Urobilinstoffwechsel, Ztschr. f. klin. Med. **114**: 765, 1930.

CARBON DIOXIDE CHANGES IN ALVEOLAR AIR AND BLOOD PLASMA OR SERUM AFTER SUBCUTANEOUS HISTAMINE INJECTION IN HUMAN BEINGS*

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HISTORY

IN THE work reviewed two main types of gastric stimulants have been used, food and histamine. Where the former has been used the Co_2 changes have usually been determined upon alveolar air. A rise in the CO_2 content of this air has been demonstrated by Higgins,[1] Dodds,[2] Bennett and Dodds,[3] Brunton and Israëls,[4] Van Slyke, Stillman and Cullen,[5] and Kauders and Porges.[6] The last two men say that this rise was found only in patients secreting an acid gastric juice.

Dodds and McIntosh[7] were unable to find an increase in plasma CO_2 in food-stimulated cases but do report an increase in the CO_2 of the red blood corpuscles. Hubbard,[8] in 1928, tested the effect of a carbohydrate high meal on patients who were suffering from "carbohydrate abnormalities" and found an increase in titratable alkalinity of the blood after such meals. Van Slyke, Stillman and Cullen[5] found an increased plasma CO_2 in most of their cases after the ingestion of food.

The results following the use of histamine as a gastric stimulant seem to fall into two classes, according to the size of the dose. Where a relatively large amount was given, there was always a fall in plasma CO_2; reports of work done on animals from Underhill and Ringer,[9] Wallace and Pellini,[10] Hashimoto,[11] Boyd,[12] and Hiller,[13] all agree on this. Small doses of histamine have been given human beings by Fonseca and de Carvalho,[14] and Delhougne,[15] who found an increase in plasma CO_2 in cases secreting hydrochloric acid in the gastric juice but not in achlorhydrias (achylias); by Katzenelbogen,[16] who found an increase in plasma CO_2 in four cases of acidosis; and by Brunton and Israëls,[4] who reported a drop in alveolar CO_2.

Feldberg and Schilf,[17] in their review of blood and alveolar CO_2 changes produced by histamine have drawn the following conclusions, which we have freely translated: "The changes in alkali reserve and hydrogen ion concentration are proportional to the amount and dependent upon the method of injection. After small subcutaneous injections of histamine, there is an increase in the P_H of the blood. After intravenous or large subcutaneous injections, there is a large decrease of CO_2 tension and of P_H of the blood; this they attributed to the increased bicarbonate output in the urine, increased lactic acid production, increased pancreatic secretion, and the direct action of histamine."

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TABLE I

CO_2 VARIATIONS ESTIMATED ON ALVEOLAR AIR AND BLOOD PLASMA OR SERUM AFTER HISTAMINE INJECTIONS OF PATIENTS WHO SECRETED HYDROCHLORIC ACID INTO THE GASTRIC JUICE

NO.	DIAGNOSIS	TIME MINUTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR CO_2 CONTENT MM. %
				CO_2 CAPACITY VOLUMES %	CO_2 CONTENT VOLUMES %	
6	Chronic cholecystitis	F.*	18-31	57.9	...	...
		30	77-87	57.9	...	...
		60	97-108	56.0	...	...
		90	64-73	58.3	...	...
8	Cholecystitis	F.	24-36	68.3	...	...
		30	70-83	70.2	...	...
		60	84-94	69.2	...	...
		90	86-95	70.2	...	...
10	Duodenal ulcer	F.	2-18	52.2	...	...
		30	61-71	70.2	...	...
		60	62-72	70.2	...	...
		90	0-10	68.3	...	...
15	Diabetes mellitus	F.	3-19	50.4	...	...
		30	12-26	52.3	...	...
		60	23-36	52.3	...	...
		90	...	65.3	...	...
38	Mitral regurgitation and functional dyspepsia	F.	40-50	39.2	...	33.7
		30	23-35	42.0	...	37.5
		60	12-23	40.1	...	41.2
40	None	F.	0-10	56.9	...	36.0
		30	24-36	56.9	...	40.5
		60	22-30	60.6	...	44.2
		90	20-32	60.0	...	40.5
42	Cholelithiasis and diabetes mellitus	F.	40-52	62.6	...	38.2
		30	72-83	55.6	...	47.2
		60	76-84	57.9	...	50.2
43	Functional dyspepsia and hypertension	F.	62-72	62.5	...	40.5
		30	74-84	62.5	...	43.5
		60	120-132	65.4	...	44.2
		90	120-127	65.0	...	44.2
44	Congenital constitutional inferior	F.	0-10	63.5	...	42.0
		30	0-7	63.5	...	43.5
		60	50-58	62.8	...	42.0
45	Duodenal ulcer	F.	32-40	59.6	...	46.5
		30	72-81	59.7	...	43.5
		60	72-80	59.7	...	45.0
		90	76-85	...	...	48.0
46	Chronic constipation	F.	4-12	59.9	...	37.5
		30	68-74	62.3	...	43.5
		60	70-76	62.3	...	45.0
		90	...	62.0	...	45.0
50	Diabetes mellitus	F.	0-8	50.4	...	36.0
		30	38-46	52.2	...	40.5
		60	28-36	54.1	...	44.2
		90	14-23	52.2	...	44.2

TABLE I (Cont'd)

NO.	DIAGNOSIS	TIME MINUTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR
				CO_2 CAPACITY VOLUMES %	CO_2 CONTENT VOLUMES %	CO_2 CONTENT MM. %
52	Irritable colon and diarrhea	F.	0-9	54.1	...	39.7
		30	20-30	59.8	...	44.2
		60	16-24	60.7	...	45.7
		90	...	61.7	...	46.5
53	None	F.	0-8	53.8	...	38.2
		30	20-26	56.7	...	42.7
		60	22-32	57.6	...	44.2
		90	...	57.6	...	44.2
54	Hypertension and arteriosclerosis	F.	78-86	56.0	...	33.0
		30	95-105	58.9	...	36.0
		60	121-129	59.8	...	39.7
		90	89-94	59.8	...	39.7
61	Chronic cholecystitis	F.	0-6	...	...	31.5
		30	32-36	...	...	40.5
		60	10-15	...	...	39.0
		90	0-8	...	...	36.0
65	Functional dyspepsia	F.	94-104	60.7	...	41.2
		30	93-104	70.1	...	42.0
		60	98-108	61.4	...	44.2
		90	68-80	60.4	...	46.5
71	Choleangeitis secondary to cholecystoduodenostomy	F.	6-23	...	...	24.7
		30	8-19	...	...	28.5
		60	62-72	...	...	30.0
73	Chronic appendicitis	F.	0-8	58.6	...	...
		30	86-95	57.6	...	...
		60	108-118	61.4	...	...
		90	88-98	62.4	...	...
76	Cholecystitis	F.	58-64	...	...	34.5
		30	6-14	...	...	35.2
		60	0-12	...	...	35.2
		90	20-30	...	...	33.7
84	Diabetes mellitus	F.	0-12	57.9	...	...
		30	29-41	59.7	...	...
		60	32-46	67.1	...	...
		90	0-14	66.4	...	...
87	Chronic pancreatitis	F.	0-10	56.7	...	...
		30	18-30	63.3	...	...
		60	38-48	59.5	...	...
		90	32-42	60.5	...	...
89	Irritable colon	F.	0-10	63.6	...	...
		30	30-40	63.6	...	...
		60	44-56	60.3	...	...
94	None	F.	20-30	55.1	...	...
		30	58-70	58.9	...	...
		60	62-74	57.9	...	...
		90	72-83	63.6	...	...

TABLE I (Cont'd)

NO.	DIAGNOSIS	TIME MINUTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR CO_2 CONTENT MM. %
				CO_2 CAPACITY VOLUMES %	CO_2 CONTENT VOLUMES %	
98	Pylorospasm	F.	10-20	49.4	59.8	...
		30	70-80	50.4	62.6	...
		60	52-62	59.8	63.6	...
		90	28-38	50.4	57.9	...
99	Duodenal ulcer	F.	42-52	54.1	60.7	...
		30	87-97	57.9	63.6	...
		60	94-104	57.0	63.6	...
		90	92-100	59.8	62.6	...
100	None	F.	0-4	55.1	55.1	...
		30	2-12	61.7	60.7	...
		60	21-31	57.0	56.0	...
		90	0-11	56.0	57.0	...
101	Neurasthenia	F.	12-26	...	56.0	...
		30	36-48	...	61.7	...
		60	48-54	61.7	60.7	...
		90	50-57	60.7	59.8	...
102	Chronic cervicitis, gall bladder disease ?	F.	0-14	55.1	51.3	...
		30	48-58	57.9	58.9	...
		60	78-90	...	...	...
		90	50-60	57.9	57.0	...
103	Psychoneurosis	F.	51-62	59.5	51.9	...
		30	115-123	59.5	61.4	...
		60	110-120	58.6	57.6	...
		90	86-96	63.3	59.5	...
104	Psychoneurosis	F.	19-30	64.5	65.5	...
		30	76-86	65.5	65.5	...
		60	108-116	65.5	63.6	...
		90	50-60	64.5	65.5	...
105	Acne rosacea	F.	0-10	62.4	60.5	...
		30	38-48	67.2	66.4	...
		60	52-63	65.5	63.6	...
		90	60-70	63.6	61.7	...

METHOD

In connection with the work done in this laboratory on phases of gastric digestion, we have made determinations of alveolar CO_2 and of plasma or serum CO_2 before and at half hour intervals after histamine stimulations of gastric secretions.

The patient, who had fasted for fifteen hours, reported to us in the morning. A duodenal tube was then swallowed and its position ascertained by the fluoroscope, with the tip in the most dependent portion of the stomach. The fasting contents were removed and control specimens of blood and alveolar air taken. Histamine was given subcutaneously (0.005 mg. per pound of body weight) and at half hour intervals afterward specimens of blood and alveolar air were taken. The gastric juice was continuously aspirated and collected over the half hour

TABLE II

CO_2 VARIATIONS ESTIMATED ON ALVEOLAR AIR AND BLOOD PLASMA OR SERUM AFTER HISTAMINE INJECTION OF PATIENTS WHO SECRETED NO HYDROCHLORIC ACID INTO THE GASTRIC JUICE

NO.	DIAGNOSIS	TIME MINUTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR CO_2 CONTENT MM. %
				CO_2 CAPACITY VOLUMES %	CO_2 CONTENT VOLUMES %	
51	Benign achylia	F.*	0-4	54.1	...	41.2
		30	0-12	57.9	...	43.5
		60	0-14	60.7	...	44.2
		90	...	63.6	...	45.7
55	Carcinoma of stomach	F.	0-8	47.5	...	33.0
		30	2-10	52.2	...	33.7
		60	0-7	58.9	...	36.7
		90	...	...	...	36.8
56	Chronic constipation	F.	0-5	53.2	...	45.0
		30	0-8	54.1	...	45.7
		90	0-6	56.0	...	49.5
57	Ptosed, spastic colon	F.	0-10	...	...	33.0
		30	0-10	...	...	34.5
		60	0-8	...	...	34.5
		90	0-10	...	...	35.3
58	Pernicious anemia	F.	0-15	...	...	34.5
		30	0-16	...	...	35.5
		60	0-13	...	...	37.5
		90	0-12	...	...	37.5
58	Second test	F.	0-10	57.9	...	35.5
		30	0-22	61.7	...	36.8
		60	0-22	61.7	...	39.0
		90	0-16	57.9	...	37.5
59	Pernicious anemia	F.	0-18	...	...	36.0
		30	0-16	...	...	33.0
		60	0-14	...	...	36.8
		90	0-14	...	...	36.8
60	Chronic appendicitis and chronic cholecystitis	F.	0-7	57.9	...	36.0
		30	0-13	57.9	...	37.5
		60	0-9	58.9	...	38.2
		90	...	60.7	...	38.2
62	Menopausal syndrome and chronic constipation	F.	0-15	49.4	...	36.8
		30	7-17	52.2	...	39.0
		60	...	67.3	...	39.0
68	Chronic alcoholic gastritis	F.	0-12	58.6	...	39.0
		30	0-10	52.8	...	38.2
		60	0-10	57.6	...	39.6
70	Pernicious anemia	F.	0-4	60.7	...	34.5
		30	0-2	54.1	...	34.5
		60	0-2	59.8	...	35.5
		90	0-2	60.7	...	34.5
83	Chronic nephritis with anemia	F.	0-16	43.8	...	...
		30	0-14	49.4	...	...
		60	0-13	40.9	...	...
		90	0-12	40.0	...	...

TABLE II (Cont'd)

NO.	DIAGNOSIS	TIME MINUTES	GASTRIC JUICE TITER %	BLOOD PLASMA OR SERUM		ALVEOLAR AIR
				CO_2 CAPACITY VOLUMES %	CO_2 CONTENT VOLUMES %	CO_2 CONTENT MM. %
88	Atonic colon	F.	0-10	57.9	...	...
		30	0-11	57.0	...	...
		60	0-10	53.2	...	...
		90	0-8	58.9	...	...
90	Carcinoma of stomach	F.	0-11	55.8	...	...
		30	0-12	58.7	...	...
		60	0-4	60.0	...	...
		90	0-16	60.0	...	...
92	Carcinoma of stomach	F.	0-12	...	...	30.7
		30	0-28	...	...	31.5
		60	0-34	...	...	32.3
		90	0-10	...	...	30.7
92	Second test	F.	0-6	56.0	...	...
		30	0-50	54.1	...	...
		60	...	57.0	...	...
107	Cardiospasm	F.	0-8	57.9	56.0	...
		30	...	59.8	57.9	...
		60	12-23	58.9	57.0	...
		90	0-8	60.7	57.9	...
108	Terminal nephritis	F.	0-8	18.3	15.5	...
		30	...	25.8	20.2	...
		90	...	18.3	18.3	...
109	Carcinoma of stomach	F.	0-11	...	52.2	...
		30	9-20	...	53.2	...
		60	11-20	...	52.2	...
		90	0-10	...	51.3	...
110	Anemia pernicious?	F.	0-13	60.7	59.8	...
		30	...	59.8	57.0	...
		60	...	58.9	58.9	...
		90	0-10	59.8	57.0	...
111	Carcinoma of stomach?	F.	0-6	56.0	56.0	...
		30	0-8	57.0	56.0	...
		60	...	57.9	57.0	...
		90	0-7	57.0	56.0	...
112	Functional gastric disturbance	F.	0-4	48.5	49.4	...
		30	0-4	52.2	50.4	...
		60	0-6	...	...	...
		90	0-4	56.0	54.1	...

periods. Töpfer's reagent was used in the estimation hydrochloric acid and phenolphthalein in that of total acidity. The plasma or serum were used interchangeably for the determination of CO_2 content and capacity; Van Slyke and Cullen's method[18] was used. For alveolar CO_2 Fridericia's indirect method[19] was used.

OBSERVATIONS

We are able to report results of tests made on 52 patients, suffering from a multiplicity of ailments. A detailed list of these pathologic conditions is

given in Tables I and II. With but few exceptions all the individuals investigated were from the Gastrointestinal Dispensary of the Johns Hopkins Hospital and represent an average group of entrants. For clearness in the analysis of results we have divided the patients into two groups. Group 1 (see Table I) contains those secreting hydrochloric acid after histamine; this group also contains 14 patients who showed no hydrochloric acid under the stimulus of an Ewald test meal but who did secrete it after histamine. Group 2 (see Table II) contains those patients secreting no hydrochloric acid, the achylias who had gastric juice with a hydrogen ion concentration between 3 and 8.

In Group 1 there are 32 patients; 17 had hydrochloric acid in the fasting juice, the remainder did not. The type of CO_2 changes following histamine

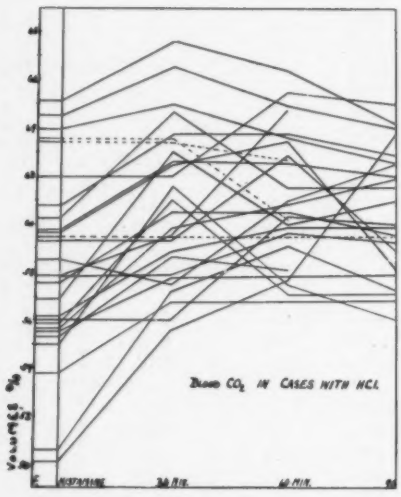

Fig. 1.

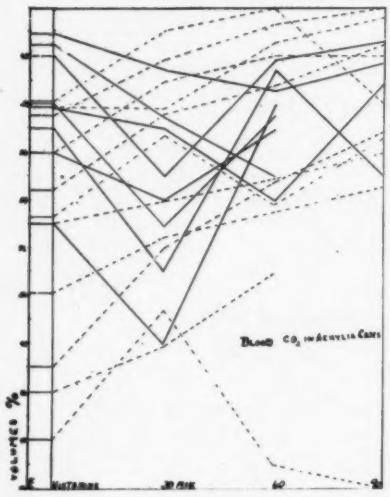

Fig. 2.

Fig. 1.—Changes in CO_2 capacity in blood serum or plasma after histamine stimulation of cases secreting hydrochloric acid in the gastric juice.

Fig. 2.—Changes in CO_2 capacity in blood serum or plasma after histamine stimulation of cases secreting no hydrochloric acid in the gastric juice.

did not seem to be influenced by the presence or absence of hydrochloric acid in the fasting juice.

A glance at Fig. 1 demonstrates the fact that during the period of secretion, the majority of those patients secreting hydrochloric acid after histamine stimulation show varying amounts of plasma and alveolar CO_2. In 25 patients there is an increase in plasma CO_2 and where the alveolar CO_2 was also determined there was a parallel increase. In two patients there was no demonstrable change; in two patients there was a fall. In three patients in whom the alveolar CO_2 alone was determined, there was a rise. There was no regularity in the time of the greatest increase but generally the peak was reached in thirty minutes; some cases continued to increase after the first rise.

In Fig. 2 of Group 2, cases secreting no hydrochloric acid after histamine stimulation, the results are more irregular than in the previous group. The most marked point is that five patients out of the twenty show a definite drop in plasma CO_2, paralleled by the alveolar CO_2. The variations in those patients who

show a poststimulation rise in plasma and alveolar CO_2 are usually less than those which secrete hydrochloric acid in the fasting juice.

Four cases of pernicious anemia are included in this group; in three there was a decrease in the alveolar or plasma CO_2 (in both if both were determined); but in the fourth there was an increase. The five cases of carcinoma show only small variations one way or the other. Two cases of terminal nephritis show an increase in the plasma CO_2. One of these, No. 108, advanced from a control CO_2 capacity of 18.3 volumes per cent to 25.8 in thirty minutes; in ninety minutes there was a return to the control level.

DISCUSSION

When this work was started it was possible to assert that in patients secreting a normal gastric juice, the effect of a meal was to increase the alveolar CO_2 tension for various periods after ingestion. Furthermore one could add the evidence of one group[6] of investigators that this rise was found only in cases secreting hydrochloric acid and not found in achylia. In their recent publication Peters and Van Slyke[20] present evidence which they feel corroborates this.

The consideration of the effects produced by histamine in human beings, however, does not allow one to make such sweeping statements as there is no agreement among the investigators. Perhaps the fact that but a small number of cases was studied by any group may be responsible for this lack of agreement.

From our work it is not possible to make any final statement as to the absolute certainty of a rise or fall in CO_2 of the plasma or alveolar air in relation to the type of gastric secretion. It is true, however, that the CO_2 of plasma and alveolar air always vary in a parallel manner.

The greater number of our acid secreting cases shows a rise in the CO_2 content but there are two definite exceptions. Furthermore, these exceptions secreted a large amount of juice of high titratable acidity. Our nonacid secreting cases do not all show a fall in their alveolar or plasma CO_2 after histamine stimulation. Some of them show a rising curve almost equal in height to that found in the acid secreting cases; others do not vary. Therefore, we cannot by any means foretell the CO_2 changes after histamine in patients not secreting an acid juice; the variation has an equal chance of being up or down.

The question as to whether the changes observed after a meal and those after histamine stimulation are comparable, is to be considered. Certainly the main action of food in normal cases is the production of hydrochloric acid. It is possible that as pancreatic secretion begins, this secondary action may greatly influence the electrolytic balance in the blood. With histamine and with the constant evacuation of the gastric contents, it is possible to lessen the degree of the second factor. Nevertheless in the majority of our cases we find the same results as those reported in all acid secreting cases after eating a normal meal, i. e., an elevation of alveolar CO_2. Therefore, although the fact that histamine may have other indirect effects upon the alveolar and the plasma CO_2 than that produced by gastric secretion, it appears that in the large part, the changes are those that follow this secretion.

CONCLUSIONS

1. Following the stimulation of gastric secretion by histamine, there is an increase in the CO_2 content of alveolar air and of plasma or serum of the great majority of patients who secrete an acid juice.

2. Following the stimulation of gastric secretion by histamine, there may be an increase or a decrease in the CO_2 content of alveolar air and of plasma or serum of patients who secrete no hydrochloric acid in the gastric juice.

3. The variations in the CO_2 content of alveolar air and of plasma or serum parallel each other after histamine stimulation of gastric secretion.

REFERENCES

1. Higgins, H. L.: The Influence of Food, Posture and Other Factors on the Alveolar Carbon Dioxide Tension in Man, Am. J. Physiol. **34**: 114, 1914.
2. Dodds, E. C.: Variations in Alveolar Carbon Dioxide Pressure in Relation to Meals, J. Physiol. **54**: 342, 1920-1921.
3. Bennett, T. I., and Dodds, E. C.: Gastric and Respiratory Response to Meals, Brit. J. Exper. Path. **2**: 58, 1921.
4. Brunton, C. E., and Israëls, M. C. G.: Interrelation of Respiration and Gastric Secretion, J. Physiol. **70**: 184, 1930.
5. Van Slyke, D. D., Stillman, E., and Cullen, G. E.: Studies of Acidosis. V. Alveolar Carbon Dioxide and Plasma Bicarbonate in Normal Men During Digestive Rest and Activity, J. Biol. Chem. **30**: 401, 1917.
6. Kauders, F., and Porges, O.: Über die Beziehugen der Kohlensäurespannung der Alveolarluft zur Pathologie der Magensaftsekretion, Wien. Arch. f. inn. Med. **5**: 379, 1923.
7. Dodds, E. C., and McIntosh, J.: Variations in the CO_2 Content of the Blood Constituents in Relation to Meals, J. Physiol. **57**: 139, 1923.
8. Hubbard, R. S.: The Effect of a Meal Upon the Titratable Alkalinity in Blood, Proc. Soc. Exper. Biol. & Med. **25**: 71, 1927-1928.
9. Underhill, F. P., and Ringer, M.: Studies on the Physiological Action of Some Protein Derivatives, J. Biol. Chem. **48**: 533, 1921.
10. Wallace, G. B., and Pellini, E. J.: Acidosis From Capillary Poisons, Proc. Soc. Exper. Biol. & Med. **18**: 115, 1920-1921.
11. Hashimoto, H.: Blood Chemistry in Acute Histamine Intoxication, J. Pharmacol. & Exper. Therap. **25**: 381, 1925.
12. Boyd, T. E., Tweedy, W. W., and Austin, W. C.: Some Effects on the Acid Base Balance, Proc. Soc. Exper. Biol. & Med. **25**: 451, 1927-1928.
13. Hiller, A.: The Effect of Histamine on the Acid-Base Balance, J. Biol. Chem. **68**: 833, 1926.
14. Fonseca, F., and de Carvalho, A.: Métabolisme et Histamine, Compt. rend. Soc. de biol. **96**: 875, 1927.
15. Delhougne, F.: Untersuchungen über die Magensaftsekretion. IV. Über die Magensaftsekretion bei Lugentuberkulose Zugleich ein Beitrag zur Frage der Histaminwirkung, Deutsches Arch. f. klin. Med. **150**: 373, 1926.
16. Katzenelbogen, S.: The Action of Histamine on the Alkali Reserve, J. A. M. A. **92**: 1240, 1929.
17. Feldberg, W., and Schilf, E.: Histamin, ed. Julius Springer, Berlin, 1930.
18. Van Slyke, D. D., and Stadie, W. C.: The Determination of the Gases of the Blood, J. Biol. Chem. **49**: 1, 1921.
19. Fridericia, L. S.: Estimation of Carbon Dioxide Tension in Alveolar Air, Hosp. Tid., Køhenh. 5.R. vii, 585, 1914.
20. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. I. Interpretations, Baltimore, 1931, Williams and Wilkins.

INTRADERMAL TEST FOR THE DETERMINATION OF MALIGNANCY*

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THE object of this paper is to present a new phase in the diagnosis of malignancy, with an attempt to establish the fact that a foreign protein of embryonal character is present in malignancy and that this protein is so characteristic that it not only produces specific precipitins, as demonstrated by the serologic test for malignancy,[1] but also shows that the fixed cells, too, are responsive to that protein, which fact is demonstrated by the allergic reaction obtained when an antigen of embryonic tissue is used. It further proves that these embryonic proteins are distinctly of a given type; that is, they are either epithelial in the case of carcinoma or of connective tissue origin in the case of sarcoma.

Long ago, many workers noticed the embryonic character of malignant cells and have even attempted to make extracts of embryos for therapeutic purposes but failed to appreciate the fact that while it is true that malignant cells are embryonic in character, the great difference lies in the type of the cell which determines its specificity; this, they have entirely overlooked. That is to say that carcinoma, being an epithelial tumor, will not respond to embryonic tissue which contains connective tissue cells, and vice versa in the case of sarcoma.

Fry[2] employed tumor tissue as an antigen for a serologic test for malignancy and, according to his own statement, the test did not prove successful in a great number of cases. The reason for it may be ascribed to the fact that tumor tissue can not be obtained in pure form to represent a given type of cell, on account of the intercellular substance and mature connective tissue cells which surround the malignant cells and which cannot be separated from them in the preparation of the antigen. Furthermore, if a malignant tumor is large enough to be used for antigenic purposes, degeneration of the tumor tissue is usually already in progress and will interfere with the reaction.

One must also keep in mind the possibility of organ specificity, as proved by Hektoen[3] and his coworkers, which is liable to give false reactions in allergically susceptible individuals. From our observations in the Clinic here at the University, a great number of patients suffering with malignant conditions have shown allergic phenomena to other proteins too. It is therefore imperative that the antigen chosen for the reaction must be of such nature that is highly allergically specialized and not within the category of nonspecific allergic susceptibilities.

Worthy of consideration is the possibility that innoculation of a patient with an antigen made of cancer tissue, enough to produce an allergic reaction, might exert antigenic influences in encouraging malignancy in an inherently susceptible

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individual. The fact that implantation of cancer tissue in man does not develop malignancy does not prove contrary to the above possibility for the simple reason that when cancer is implanted, degeneration of the implant rapidly takes place on account of the lack of blood supply, and therefore the antigenic influence does not assert itself; while when an extract of purely epithelial cells is introduced, their characteristic protein might act immediately, as demonstrated by the allergic reaction.

As seen from the above, the preparation of an antigen for the purpose of determining malignancy must be of such nature that it will not be affected or marred by any of the above enumerated obstacles. In order to do so, cells of purely embryonic character must be resorted to, and accordingly the following tissues have been adopted and found to give the desired results. In the case of carcinoma, pancreas and submaxillary glands of very young embryo calves were used; for sarcoma, Wharton's jelly and the bone marrow of young embryos were utilized, as per the following technic.

Preparation of Antigen, Preliminary Steps.—It is essential that in the preparation of the antigen, both for carcinoma and sarcoma, all glassware and other utensils used be chemically clean, sterile, and of neutral reaction.

Preparation of the Antigen for Carcinoma.—Mammalian embryos are used (calves, sheep, pigs). They must not be in a later stage than the second month of fetal life. This is readily recognized by their relative smallness, and by the smoothness of the skin (for instance, there is no formation of hair). In securing these embryos, the abdomen must be opened under sterile conditions. The pancreas and submaxillary glands are dissected out, and placed in a sterile dish. A hypotonic salt solution is poured over these, and if possible, allowed to freeze, the object being to permit an easier removal of the fibrous capsule. The capsule and the ducts, etc., are then removed by careful manipulation with small tissue forceps. The epithelial tissue is picked out. It is placed in a mortar in which sterile copper gauze is inserted (to facilitate the maceration) and macerated in physiologic salt solution. At this point, the cells suspended in salt solution are centrifuged and the supernatant salt solution is discarded. The epithelial cells are placed in a porcelain dish and dried at a temperature of 75° C. for a few minutes until the water separates, which should then be poured off, leaving the cells in a state of doughy consistency. These are then covered with ether, shaken up, the ether decanted and the cells placed at room temperature for a few minutes to allow the remaining ether to evaporate. It is then rubbed up with twenty-five times its volume of one-tenth normal sodium hydroxide, by adding the sodium hydroxide slowly, one cubic centimeter at a time, and macerating it vigorously until the cells are thoroughly rubbed up, so that on standing for awhile the least amount of the remaining cells fall to the bottom. It is placed at room temperature for twenty-four hours, after which it is centrifuged at a low speed for ten minutes, the supernatant fluid is then pipetted off and neutralized with one-tenth normal hydrochloric acid, drop by drop, until it is brought to neutrality, at a P_H of seven.

Recent experiments have shown that embryonic liver may be used to advantage in the preparation of the antigen for carcinoma, since a larger amount

TABLE I

CASE	GRUSKIN INTRADERMAL	BIOPSY OR AUTOPSY	OPERATION	CLINICALLY OUTSPOKEN
H. F.	Carcinoma positive	Carcinoma of breast	Carcinoma of breast	
B. K.	Carcinoma negative			Advanced, active bilateral pulmonary tuberculosis
C. J.	Carcinoma positive	Carcinoma, probably bronchiogenic	Carcinoma of larynx	
R. S.	Carcinoma positive	Extensive adenocarcinoma	Adenoma of ovary	
J. R.	Carcinoma positive	Fibroadenoma, malignant change		
M. P.	Carcinoma negative			Advanced pulmonary tuberculosis, laryngeal tuberculosis
M. C.	Carcinoma positive	Chronic cystic mastitis		
E. B.	Carcinoma negative	Chronic cystic mastitis	Chronic cystic mastitis	
E. C.	Carcinoma negative			Syphilis
Mrs. D.	Carcinoma positive			Confirmed by x-ray
F. T.	Carcinoma negative			Bilateral pulmonary tuberculosis
F. H.	Sarcoma positive	Endothelioma of bone that has spread to surrounding soft structures		
V. B.	Sarcoma positive	Melanosarcoma	Melanotic sarcoma	
P. W.	Carcinoma negative			Diabetes
J. L.	Carcinoma positive	Fibroadenoma undergoing malignant change	Fibroadenoma of breast	
V. B.	Carcinoma negative			Incipient tuberculosis
R. W.	Carcinoma negative			Nephritis
H. P.	Carcinoma positive			Carcinoma of prostate, with metastasis to lungs and bones
A. B.	Carcinoma negative			Syphilis
T. W.	Carcinoma positive	Alveolar carcinoma of appendix	Acute appendicitis with pelvic abscess	
L. A.	Carcinoma negative			Early pulmonary tuberculosis

TABLE I (Continued)

CASE	GRUSKIN INTRADERMAL	BIOPSY OR AUTOPSY	OPERATION	CLINICALLY OUTSPOKEN
S. P.	Carcinoma negative		Abscess in tempero-mandibular region	
M. A.	Carcinoma positive	Squamous cell carcinoma	Carcinoma of cervix	
R. W.	Carcinoma negative			Early tuberculosis
W. S.	Carcinoma positive			Carcinoma of pelvic organs with metastasis to peritoneum
L. S.	Carcinoma negative			Syphilis
E. L.	Sarcoma positive	Periosteal sarcoma	Sarcoma of iliac bone	
H. M.	Carcinoma positive	Adenoma of stomach undergoing malignant change	Gastric malignancy	
L. M.	Carcinoma negative			Fibroid tuberculosis
J. W.	Carcinoma positive	Carcinoma		
R. J.	Carcinoma positive	Adenoma undergoing malignant degeneration	Carcinoma of gall bladder	
L. B.	Carcinoma positive	Necrotic tissue, malignant	Carcinoma of cervix	
E. G.	Carcinoma negative			Bilateral pulmonary tuberculosis
R. M.	Carcinoma negative	Early Hodgkin's disease		
C. E.	Carcinoma positive			Extensive carcinoma of bladder (infiltrating)
A. S.	Carcinoma negative			X-ray: tuberculosis of lung
M. B.	Sarcoma positive	Retroperitoneal sarcoma		
E. H.	Carcinoma negative			Active pulmonary tuberculosis
B. E.	Carcinoma positive	Suspicious malignancy	Possible carcinoma of vagina	
B. G.	Carcinoma positive		Considered inoperable	Carcinoma of breast
A. B.	Carcinoma negative			Bilateral pulmonary tuberculosis
S. A.	Carcinoma positive		Carcinoma of prostate	
H. G.	Carcinoma negative			Diabetes

TABLE I (Continued)

CASE	GRUSKIN INTRADERMAL	BIOPSY OR AUTOPSY	OPERATION	CLINICALLY OUTSPOKEN
E. C.	Carcinoma negative	Chronic interstitial adenitis		
F. T.	Carcinoma positive	Epithelioma		
K. H.	Carcinoma negative			Bilateral pulmonary tuberculosis
J. L.	Carcinoma positive	Labial carcinoma	Labial carcinoma	
S. R.	Carcinoma positive	Metastatic carcinoma		
A. B.	Carcinoma positive		Carcinoma of cecum and sigmoid	
M. B.	Carcinoma negative		Lung abscess	
L. K.	Carcinoma negative			Pneumothorax case; chronic prostatitis; duodenal ulcer
A. H.	Sarcoma positive; Carcinoma negative	Spindle cell sarcoma, Hodgkin's disease		
H. M.	Carcinoma negative			Syphilis
J. A.	Carcinoma positive	Squamous cell carcinoma	Necrosis external ear; unhealed radical mastoid	
S. G.	Carcinoma negative	Simple colloid goiter with considerable fibrosis	Adenoma of thyroid	
J. M.	Carcinoma positive	Rodent ulcer		
Mrs. H.	Carcinoma positive	Mucinous carcinoma, cervical endometrium		
Mrs. C.	Carcinoma positive	Adenocarcinoma of rectum, resected		
Mrs. M.	Carcinoma positive	Squamous cell carcinoma of the forehead. Diabetic		
Mr. McC.	Carcinoma positive	Teratoma (mixed carcinoma-sarcoma) mucinous type		
Mr. G.	Carcinoma positive	Adenocarcinoma of the prostate		

Twenty cases of normal individuals gave negative reactions.
Four cases of diabetes gave negative reactions.
One case of polycythemia gave a negative reaction.

of material may be obtained with greater ease. From the embryonic liver the cells are obtained in the following manner: the whole liver is cleaned of the capsule and washed with water to remove as much blood as possible. It is then placed in an Erlenmeyer flask with water and shaken vigorously until all the cells

are separated from the fibrous tissue. The cells and water are then centrifuged at high speed for five minutes, the water poured off and the cells washed repeatedly until all traces of blood are removed. For the intradermal antigen the wet cells are rubbed up with twenty volumes of one-tenth normal sodium hydroxide, allowed to stand for twenty-four hours, and then neutralized and prepared as in the case of the embryonic pancreas. For the serum antigen, the cells are placed in five volumes of acetone for twenty-four hours, then centrifuged, the acetone poured off and the cells dried in a desiccator. They are then rubbed to an impalpable powder and mixed with alcohol as described in the case of the embryonic pancreas.

Antigen for Sarcoma.—A mixture of Wharton's jelly and red bone marrow of calf embryo is macerated in a mortar, to which two volumes of salt solution are added. This is placed in a porcelain dish at a temperature of 75° C. until the water separates, which should then be poured off, leaving the cells in a state of doughy consistency. These are then covered with ether, shaken up, the ether decanted and the cells are placed at room temperature for a few minutes to allow the remaining ether to evaporate. It is then rubbed up and with one-tenth normal sodium hydroxide, in the proportion of one gram of cells to 25 c.c. of sodium hydroxide. The sodium hydroxide is added slowly, one cubic centimeter at a time, macerating the cells vigorously until they are thoroughly rubbed up, so that on standing for awhile the least amount of the remaining cells fall to the bottom of the tube. The tube containing the solution is placed in the ice box for twenty-four hours after which the supernatant fluid is pipetted off and neutralized with one-tenth normal hydrochloric acid, added drop by drop, until it is brought to neutrality, at a P_H of seven.

After being neutralized, the antigens are then placed in small vaccine bottles and kept in a cool, dark room, and are ready for use.

The Test Proper.—Two-tenths of a cubic centimeter of the antigen is injected intradermally with a very fine needle. Care must be taken that the injection should not be forced, so that no false pseudopods will be formed. In positive cases, a slight area of inflammation with pseudopod formation appears within fifteen minutes. In negative cases, no such reaction takes place. It is advisable to use a control of plain physiologic salt solution with each test. The control must always be negative, showing no inflammation and no pseudopods.

Precautions.—In patients who are emaciated and where the skin assumes a paper-like thinness so that a correct intradermal test is impossible to be performed, it is advisable to resort to the serologic test described by the author in the *American Journal of the Medical Sciences,* April, 1929. For in these cases on account of the irregularity in the contour of the skin, one might mistake the natural appearance for pseudopods, and, vice versa, the pseudopods might not be easily distinguished. It is also advisable not to perform the intradermal test on patients with septic temperatures or jaundice, nor soon after x-ray, radium treatment, or anesthesia, as false reactions might take place in such cases.

Summary.—An intradermal test for the determination of malignancy has been introduced. The antigen is made up of purely embryonic tissue obtained from pancreas and submaxillary glands of embryonic calves, in the case of carcinoma; and of Wharton's jelly and red bone marrow in the case of sarcoma.

The theory is advanced that the characteristic embryonic protein is carried not only in the blood stream but also finds response in the fixed cells, as expressed by the allergic reaction. The correct results obtained in a great number of positive and negative cases have been demonstrated, so that we feel justified in publishing this preliminary report. It is of interest that in 116 cases of intradermal tests done on students under the auspices of Professor Fanz, head of the Department of Pathology of Temple University School of Medicine, the following results were obtained:

One hundred and seven students gave no reaction.

Eight students gave a slight reaction to carcinoma, and of these, the following history was obtained: 1 had a maternal history of malignancy for three generations; 1 had a maternal history of malignancy for two generations; 6 had a family history of malignancy for one generation.

One student gave a slight reaction to sarcoma and no reaction to carcinoma. He had a paternal history of sarcoma.

REFERENCES

1. Gruskin, B.: A Serum Test for the Diagnosis of Cancer, Based on a New Theory of Etiology, Am. J. M. Sc. **177**: 476, 1929.
2. Fry, H. J. B.: Reaction de Botelho dans le sero-diagnostic du Cancer, Bull. Assn. franç. Etude du Cancer **14**: 52, 1925.
3. Hektoen, Ludvig, and Welker, William H.: The Precipitin Reaction of Fibrinogen, J. Infect. Dis. **40**: 706, 1927.

LABORATORY METHODS

TECHNICAL CONTRIBUTIONS TO THE STUDY OF GASTROINTESTINAL MOTILITY*

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A. ARRANGEMENT AND ADJUSTMENT OF MANOMETERS

THE application of the tandem-balloon method, with the use of large numbers of balloons so placed as to correlate the activity of different levels of the gut in a serial manner, has been limited by difficulties which are self-evident. When water manometers are used as recording apparatus, the problem is presented of arranging them so that their writing points are in vertical alignment. To overcome this difficulty a multiple manometer clamp has been devised which allows the entire set of manometers to be moved as a unit without disturbing the relationship of the manometers to each other, and which allows larger numbers of manometers to be clamped in a small space than is possible with other methods (Fig. 1-*A*).

Individual manometers can be raised or lowered, or removed from the clamp without disturbing the others. In order to remove a manometer, it is necessary to lift off the cap and the writing arm, after which the manometer can be drawn downward by releasing the screw which clamps it. It is advisable in setting up manometers in the clamp to place those which will write on a lower level on the side of the clamp next to the recording surface. In this way there is less conflict between writing arms as they pass to the drum. The clamp is conveniently set with its long axis at an angle of 20° to 30° to the recording surface.

A clamp stand was constructed for holding the manometer clamp, by bolting a rod into a slotted bar so that the rod could be moved in the slot, and brought into any desired position. The slotted bar was in turn bolted into an ordinary wood clamp, so as to rotate about its point of fixation to the latter (Fig. 1-*B*). The wood clamp is fastened to the table or kymograph stand, and the entire battery of manometers can be swung into or out of position.

With this arrangement of manometers the usual methods of holding the writing arms against the drum are impracticable. A simple guard was formed by gluing in a vertical position from the manometer cap a flexible strip of celluloid which could be brought to bear suitable pressure against the writing arm. A more permanent and efficient arrangement is now in use (Fig. 2). A drawn glass tube is fastened into a coiled spring of just sufficient strength

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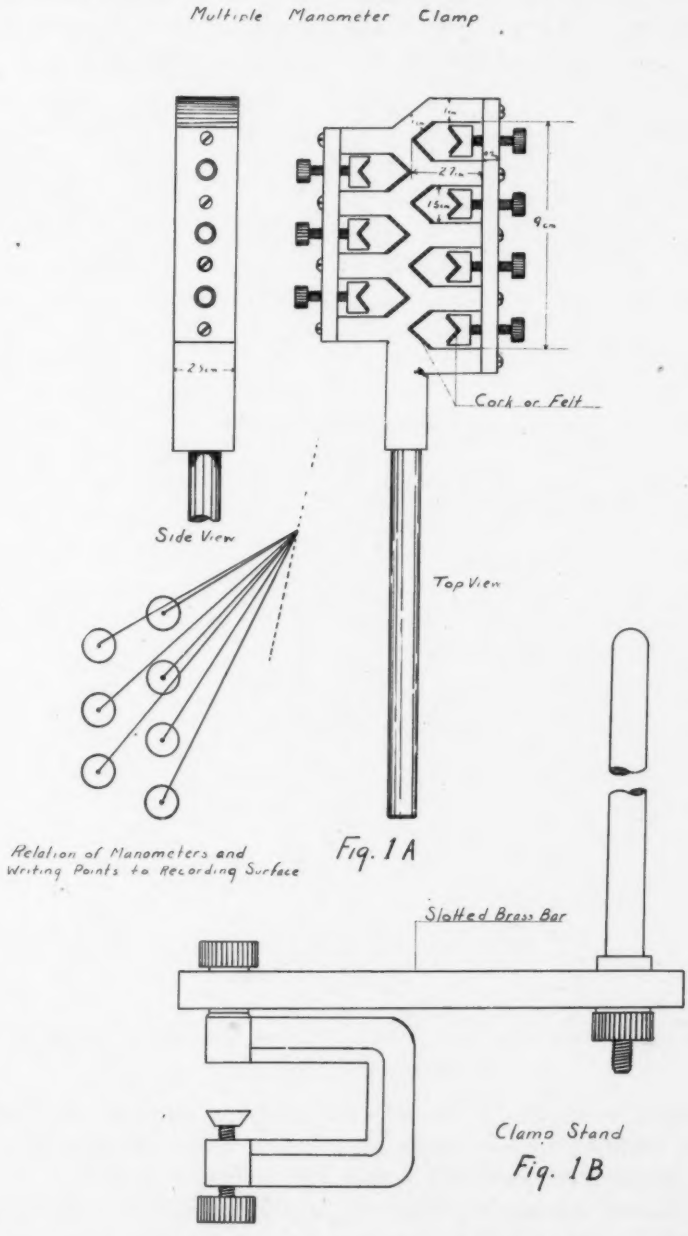
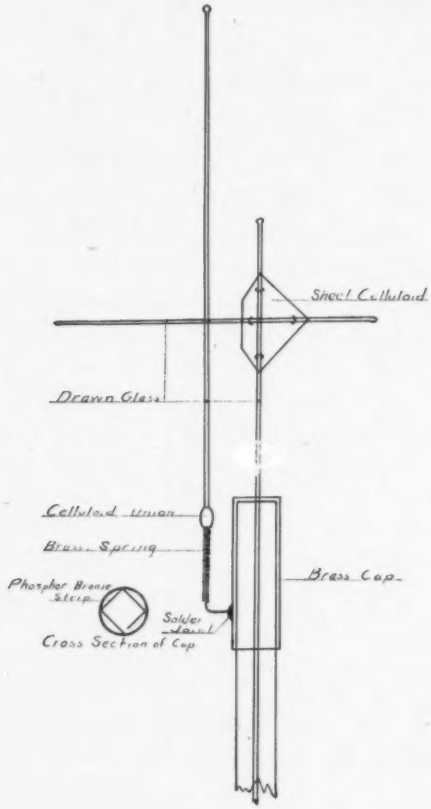
to support the glass tube vertically. The spring is soldered to the side of the manometer cap. The guard is brought into position against the writing arm by rotating the cap. To prevent rocking of the cap, with disturbance of the guard, the sides of the cap are made much longer than is customary. The



caps now in use are 4 cm. tall. To prevent rotation of the cap, with change of tension against the writing arm, a thin sheet of phosphor-bronze was bent at angles as shown in the cross section, and inserted within the cap before forcing the latter on the manometer head. Caps were made to fit the largest manometer

loosely, to allow for insertion of this strip. This obviates the necessity of having caps made in the shop to fit each manometer.

Union between the glass writing arm and the float-extension was made by means of a sheet of celluloid, cut and perforated as shown in Fig. 2. Adjustment of the writing points to a vertical line is easily accomplished with the manometers set up in the multiple clamp, by sliding the writing arm through the celluloid union. Vertical spacing is done in a similar manner.



Details of Manometer Cap and Guard

Fig. 2

B. A METHOD FOR SIMULTANEOUSLY RECORDING CHANGES IN DIAMETER AND LENGTH

The method is applicable to the unanesthetized animal, as well as to the animal with viscera exposed under anesthesia. Both changes in length and changes in diameter are recorded within the lumen of the gut.

The apparatus consists essentially of a plunger which moves freely within a protecting tube, one end of the plunger being held against the closed end of an intestinal pouch, while the other end, projecting outside the pouch, transmits movements of the plunger to an air-filled balloon lying in a cylinder, and leading to a recording water manometer. Balloons are tied at intervals on the outside of the tube which carries the plunger, and are led to water manometers (Fig. 3).

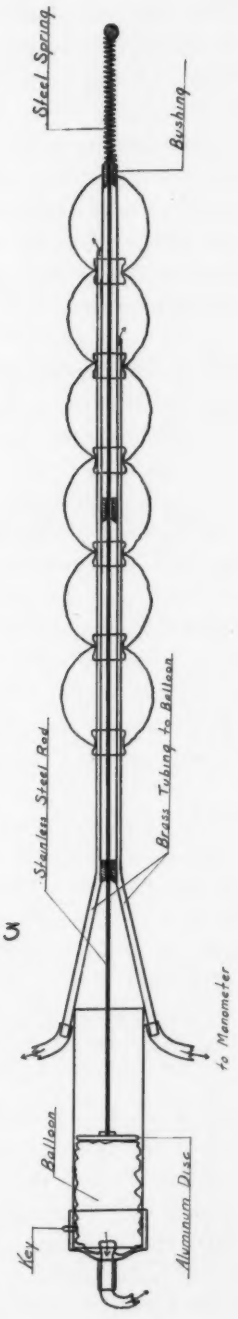

Steel Spring
Bushing
Brass Tubing to Balloon
Stainless Steel Rod
to Manometer
Balloon
Aluminum Disc
Key

Fig. 3

The plunger itself is constructed of stainless steel, about 2 mm. in diameter of cross section, and is made sufficiently longer than its protecting tube to record the expected activity. The diameter of the apparatus is kept as small as possible, by using small tubes, and by flattening the balloon leads against the plunger tube. The balloon leads run under collars which afford a smooth surface for tying balloons. Solder is run in under the collars to make an air-tight seal between balloon compartments.

The end of the plunger which impinges against the wall of the gut is capped with a small metal ball. A coil spring is compressed between this ball and the end of the plunger tube. The spring is of sufficient strength to hold the plunger fully extended when outside the gut, and is not fastened at either end. When the balloon is distended within its cylinder, the spring may be grasped between two fingers and completely compressed against the end of the plunger tube without moving the plunger.

A number of technical difficulties are encountered in using this apparatus. When the plunger becomes inactive during a tracing, after progressive lowering of the writing level, the explanation may be found in a leak in the balloon lying within the cylinder which has caused the balloon to collapse away from the piston. On the other hand, there may have been a relaxation of the gut so that the force applied against the end of the plunger is no longer sufficient to move it. The tracing does not differentiate between the two situations. However, by cutting away completely, or slotting the plunger tube as it enters the cylinder, the plunger is rendered visible, as shown in the sketch, and the position of a scratch on the plunger is a valuable guide. If the pouch has relaxed, the plunger will be found completely extended; if the balloon has leaked, the plunger may be in its original position. To allow for moderate relaxation of the pouch, the apparatus is inserted so that the plunger is about half extended at the start.

The coil spring which is slipped over the extended portion of the plunger serves two purposes, one of which is theoretical. The spring serves to keep the plunger extended, allowing the balloon within the cylinder to be distended under lower pressure. It was also thought that it might protect the plunger from movement under the influence of such activity as peristalsis, which could conceivably be of such intensity that the moving ring of constriction would grasp the unprotected plunger, and sweep it downward. It was thought that the coils of the spring would be moved instead, if this occurred, and plunger movement diminished.

For use in the exposed intestine, a perforated ball or a small metal ring is attached to the extended end of the plunger, by means of which the plunger is anchored in place with a stitch carried through the intestinal wall. This obviates the necessity of preparing an intestinal pouch for acute work.

In inserting the apparatus, the cap and the balloon are removed from the cylinder. When the plunger is in position against the end of the pouch, the balloon is inserted within the cylinder, the cap slipped on, and all balloons inflated. In Fig. 3 six tandem balloons are shown. The sketch has been simplified by omitting the side tubes leading to all but two of these. With this number of balloons almost any length of pouch can be studied without recon-

struction of the apparatus. Most of our pouches are of such length that only three or four of the tandem balloons lie in the pouch when the plunger is inserted to recording position.

In order to minimize mechanical errors due to lateral movements of the animal during the course of a tracing, the apparatus is held in position by means of a clamp made from a large burette clamp by placing two swivel joints, with their axes of rotation at right angles, in the arm of the clamp. The apparatus thus rotates freely about a fixed point, and the axis of the plunger is automatically aligned with the long axis of the pouch. The clamp is applied to the cylinder, and secured in an ordinary clamp stand which is bolted to the table.

C. A SIMPLIFIED ENTEROGRAPH

The apparatus differs from a number of devices designed for the same purpose in its simplicity and its adjustability. The materials required are a frog lever and some stiff wire. One length of wire is passed through the pivot of the frog lever, and fastened in place by the set screw, after being bent at right

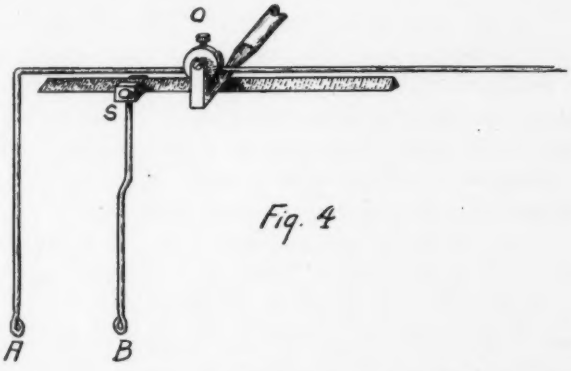


Fig. 4

angles. Another, shorter piece, is soldered to the fulcrum of the lever. The ends of the wire are bent to form eyes for fastening to the intestinal strip or ring. The tracing may be taken directly from the end of the long arm of the movable lever, or transmitted to a second lever for recording.

A modification of this apparatus which has the advantage of wide adjustability without change of leverage is shown in Fig. 4. The distance between the movable end *A* and the fulcrum *O* may be kept constant while adjustments are made by shifting the position of the passive arm *B* on the cross piece. The sleeve *S* which carries this arm is open above so that it slides freely over the entire length of the cross piece. The latter is soldered to the fulcrum of the frog lever at such a point that the cross piece and the movable arm of the lever do not lie in a vertical plane. If this is not done the lever and the cross piece will be brought into contact with high activity. The arms *A* and *B* are made sufficiently long to carry the gut, in position between them, below the surface of the bath. In working on large numbers of segments, or on the exposed gut in situ, it is usually more convenient to transmit the movements of the lever to a second lever by means of a string for taking the record.

The writers wish to express their gratitude to Mr. Gus Lutz, of the Mechanical Department, whose practical suggestions were invaluable.

FRAGILITY OF RED CORPUSCLES AND ITS DETERMINATION IN CLINICAL WORK*

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INTRODUCTION

RED corpuscles mixed with water or hypotonic solutions disintegrate and show hemolysis. Experiments made during the last years proved that the old opinion which attributed the cause of this process to some changes of the membrane of corpuscles is untenable. The pellicle is permeable to hemoglobin and can disappear without causing a hemolysis. Hemoglobin is probably combined with some substances in the corpuscles chemically, and this compound is decomposed by all agents producing hemolysis. The ease with which the disintegration of the protoplasm of red cells takes place may be called their fragility.

Hamburger, in his recent essay concerning the methods for the determination of the resistance of red corpuscles to hypotony,[1] came to the conclusion that the clinical application of these methods was not successful. Such a conclusion is not surprising because the methods used were either too complex or gave inexact and incomparable results. In most cases a threshold of hemolysis, that is a concentration in which no hemolysis is observed, but a slight decrease of which leads to hemolysis, was determined. Hamburger pointed out rightly that the results of this method depended upon the volume of the solutions and the size of containers used. Moreover, traces of hemoglobin in the solution were discovered with a different accuracy by different observers. To these two objections we may add two more.

The determination of the threshold of hemolysis gave only an account of the state of the corpuscles with the greatest fragility. A change of conditions however could influence the corpuscles with a median fragility more than other corpuscles. Sometimes the threshold of hemolysis is found alike in different men, whereas the fragility of their red blood cells with median properties differs by more than 100 per cent (see below). Moreover in all methods used up to the present only the volume of the corpuscles and not their hemoglobin content was considered. As the water content of the corpuscles may vary, the hemolysis may be found alike whereas the fragility may be different.

Among the methods used for the determination of the resistance of corpuscles to hypotony, Arrhenius' method is the only one which does not depend upon the volume of solutions and containers and permits to observe the changes in corpuscles of different fragility. The author had the opportunity to work in Arrhenius' laboratory and to use his method in most experiments. The application of this method to clinical work requires, however, some improvements.

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First, one has to avoid taking too much blood from a patient. Second, a prolonged centrifugalization before testing should be avoided, because every centrifugalization decreases the resistance of corpuscles to hypotonic solutions.[2] Third, it is well known that salt solutions change the fragility of corpuscles; therefore, one has to avoid a too prolonged action of any salt on corpuscles before they are put in a hypotonic solution. Fourth, not the volume of corpuscles but their hemoglobin content should be considered in estimating the number of hemolyzed corpuscles. All these requirements are fulfilled in the following method used by the author in his experiments on the influence of sunlight on men.

METHOD

Hypotonic Solutions.—To avoid coagulation of blood hypotonic solutions of sodium sulphate are used. Four solutions are prepared for a whole series of experiments at once in the amount of 500 c.c. by diluting a standard solution with distilled water. The standard solution contains 16.77 gm. of anhydrous sodium sulphate in 1000 c.c. To both, water and the standard solution, a sufficient amount of the solutions of monobasic (1.5 per cent) and dibasic sodium phosphate (3.5 per cent) is added to adjust P_H to 7.3. The solutions are prepared by mixing the following volumes of the standard solution with water:

	STANDARD SOLUTION	DISTILLED WATER
First sol. (0.70 per cent)	209.5 c.c.	290.5 c.c.
Second sol. (0.64 per cent)	191.5 c.c.	398.5 c.c.
Third sol. (60 per cent)	179.5 c.c.	320.5 c.c.
Fourth sol. (0.54 per cent)	164.5 c.c.	335.5 c.c.

The solutions are kept in glass stoppered bottles in a refrigerator, and once in two weeks the P_H of the solutions is tested and adjusted if necessary to 7.3. With a pipette, 5 c.c. of each solution are poured into four centrifuge tubes, closed with corks. Into the fifth tube (also closed with a cork) 5 c.c. of distilled water are poured. All solutions should have the temperature of the room before adding blood.

Blood is taken either from a finger or from the cubital vein. In the first case one Sahli pipette full of blood (0.02 c.c.), in the second case one drop of blood, is introduced into each of the 5 centrifuge tubes, and the latter are shaken once and left standing for half an hour (to settle the osmotic equilibrium), and then shaken once more and centrifuged for two minutes to sediment the corpuscles.

The estimation of the degree of hemolysis produced by each solution may be made by means of a colorimeter comparing the color of the supernatant liquids from tubes 1 to 4 with the color of the liquid in the fifth tube. This method is however not exact in our case because of the small amount of liquids and of too great a difference between the colors of the liquids. More accurate is the following method used by the author which does not require any expensive colorimeter:

This method rests on the same principle as the Sahli method for the estimation of hemoglobin. The color of the supernatant liquids in the second to fourth tubes is compared with the color of the properly diluted completely hemolyzed solution in the fifth tube. After the dilution this solution should have a fainter tint than the solutions to be estimated. This is achieved by diluting it in the following way:

× 308.5 c.c.

For fourth and third solutions: 4 c.c. of solution 5 plus 16 c.c. distilled water. This diluted solution is diluted further:

For third and second solutions: 5 c.c. of it plus 5 c.c. water.

For second solution*: 5 c.c. of it plus 15 c.c. water.

After the dilution is made, exactly 2 c.c. of the supernatant liquids from one of the solutions to be estimated (second to fourth) are poured into a specimen vial (5 cm. high, 2.5 cm. wide) graduated in cubic centimeters with divisions of 0.2 cm. Into a similar vial which needs no graduation, the corresponding diluted solution is poured. The height of the liquids in the vials should be the same so that the amount of the liquid in the not graduated vial should be adjusted to the amount in the graduated one. Both vials are standing on a white porcelain plate (6 inches square) at the posterior edge of which a mirror (the same size) is fastened which reflects light from the window onto the vials (the observer is sitting with his back to the window). Then by means of a pipette with a rubber ball water is added by drops to the solution to be tested until the color of the liquids in both vials becomes equal. Not only the color of the column of the liquids but also that of their menisci is considered. The height of the meniscus in the graduated vial after the color of the liquids has been considered equal, expressed in cubic centimeters (according to the graduation) and multiplied by 10 represents the percentage of hemolysis in case the fourth solution is tested. Multiplied by 10 or 5 (according to the dilution used) it represents the percentage of hemolysis in the third solution, and multiplied by 5 or two and a half the percentage in the second solution.

The hemolysis in the first solution is very slight, and it should be estimated in the following manner.

Into one specimen vial, 4 c.c. of this solution, into the other vial 4 c.c. of water are poured, and then by means of a pipette, 1 c.c. divided into 0.01 c.c., the completely hemolyzed solution from the fifth tube (1 c.c. left from the dilution) is added to water, until the color of the liquids and their menisci in both vials becomes equal. The corresponding percentage of hemolysis is the following:

The amount of solution from the fifth tube added in c.c.	0.01	0.02	0.04	0.08	0.12	0.16	0.20	0.24
Hemolysis %	0.2	0.5	1	2	2.9	3.8	4.8	5.7

RESULTS

Average Fragility.—Supposing that the hemolysis, in percentage, is found in the first solution 1.2, that in the second solution 11, that in the third 26 and that in the fourth solution 62, the average fragility of red cells is 25. It expresses the fragility not only for a certain kind of cells, that is, for the most resistant or for the most fragile ones, but the average of all cells.

In Table I the hemolysis in the above solutions and the average fragility is given for a normal man, in Table II for different men at 11 A.M. in December, 1931 (Tucson, Arizona).

From Tables I and II it may be concluded that the estimation of hemolysis produced by one hypotonic concentration, for instance the determination of a threshold of hemolysis, does not give us an idea about the average fragility of

*The second and third solutions may be therefore compared twice: first with a less and second with a more diluted solution 5.

TABLE I

NUMBER OF HEMOLYZED CELLS IN PERCENTAGE FOR A NORMAL MAN

DATE, TIME	NA_2SO_4: 0.70%	0.64%	0.60%	0.54%	AVERAGE FRAGILITY
3 P.M. Dec. 15, 1930	2.5	19	40	85	36.6
3 P.M. Dec. 17, 1930	2.5	23	60	85	42.6
11:30 A.M. Dec. 19, 1930	2.7	28	51	87	42.1
11:30 A.M. Jan. 7, 1931	4	27	58	90	44.7
11 A.M. Feb. 19, 1931	1.7	12	32	73	29.7
10 A.M. March 11, 1931	2.5	11	36	88	34.3

TABLE II

NUMBER OF HEMOLYZED CELLS IN PERCENTAGE FOR DIFFERENT NORMAL MEN

AGE	NA_2SO_4: 0.70%	0.64%	0.60%	0.54%	AVERAGE FRAGILITY
25	3	28	64	95	47.5
25	2	21	64	92	44.7
25	2.5	26	52	86	41.6
25	1.8	22	54	92	42.4
28	2.5	23	58	93	44.1
28	2.5	23	60	86	42.9
30	3	28	64	94	47.2
31	2	30	60	83	43.7
35	1.8	20	59	96	44.2
35	1.8	23	49	82	38.9
42	2	21	48	82	38.2
43	2	26	49	74	37.7
50	1.8	20	40	76	34.4
55	2.2	22	50	65	34.8
60	3	27	54	62	34
Average for the age 25 to 40	2.3	24.4	57.4	89.9	43.7
Average for the age 40 to 60	2.2	23.5	48.2	71.8	35.8

The average fragility of red cells for all ages is about 40, its maximum is 47.5 and its minimum is 34

TABLE III

NUMBER OF HEMOLYZED CELLS IN PERCENTAGE

EXPERIM. NO.	FRAGILITY DETERMINED	NA_2SO_4: 0.70%	0.64%	0.60%	0.54%	AVERAGE FRAGILITY
1	Before the exposure to sunlight	2.7	28	51	87	42.1
	After the exposure for half an hour	0.9	11	36	75	30.7
2	Before rise of temperature	2.5	15	36	73	31.6
	After the maximum temperature was reached	1	5	17	40	15.7

red cells. The hemolysis in one concentration can be alike, while the average fragility may differ quite distinctly. From Table II it is even seen that the degree of hemolysis in small concentrations, and therefore the threshold of hemolysis, is often greater in some men than in others while this degree in stronger concentrations, and also the average fragility are greater in the former than in the latter. It is evident therefore that only the whole curve of hemolysis can give us a correct idea about the changes of the fragility of red cells. To obtain the curves we may plot on the abscis axis the concentration of Na_2SO_4 and on the ordinate axis the hemolysis in percentage. The variation of fragility in a single man is expressed in the curves of Fig. 1.

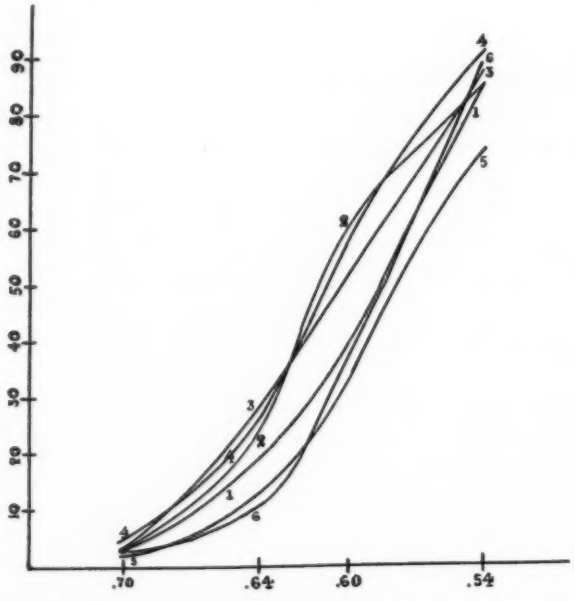

Fig. 1.—The curves of fragility of red corpuscles in a normal man. *1*, 3 P.M. Dec. 15, 1930. *2*, 3 P.M. Dec. 17, 1930. *3*, 11:30 A.M. Dec. 19, 1930. *4*, 11:30 A.M. Jan. 7, 1931. *5*, 11 A.M. Feb. 19, 1931. *6*, 10 A.M. March 11, 1931.

The above differences in fragility are evidently produced by some changes in protoplasm but also in the medium surrounding the red cells. How markedly the outer conditions may influence the fragility is seen from Table III where the results of the experiments are given in which men were submitted to a general insolation or to an increase of the temperature of body brought about by an electric current.

In case a curve of hemolysis is wanted four concentrations represent a minimum for the drawing of a curve. For clinical work, however, only the fragility, that is an average of degrees of hemolysis is necessary. In this case the concentration 0.70 per cent influences only little the result and can therefore be omitted.

COMMENT

The method of the determination of the fragility of red blood cells presented in this paper is the only method which yields a correct estimation of the resistance of red blood cells to hypotony using two to five drops of blood. In spite of its simplicity this method is more

exact than former methods and its results do not depend upon the volume of the solutions nor the size of the containers used as is the case in the method of the determination of the threshold of hemolysis. Like Arrhenius' method, the method presented in this paper gives a complete curve of the degree of hemolysis depending upon various concentrations of a salt, or the average fragility of red corpuscles. Some results presented show that only the whole curve of fragility or the average fragility give us a correct idea about the changes of the fragility of red blood cells, because the determination of hemolysis produced by one concentration of a salt solution only, and therefore that of the threshold of hemolysis, give us just the fragility of one kind of corpuscles. For instance in the case of the determination of the threshold only, the fragility of the least resistant corpuscles is tested, while the corpuscles with the greatest resistance or of medial properties react often quite differently. It is therefore not surprising that sometimes the threshold of hemolysis is the same in two specimens of blood while the average fragility of their red cells differ from each other by 100 per cent. Sometimes the threshold of hemolysis may be found at a greater concentration, while the average fragility decreases.

The method presented in this paper has the following advantages in comparison with Arrhenius' method: (1) It requires much less blood (10 to 50 times less). (2) The corpuscles are not centrifuged before they are exposed to the action of hypotonic solutions; every centrifugalization decreases the resistance of red cells to hypotony as it was shown by the author's former experiments (l. c.). (3) It does not require a washing and keeping of red cells in salt solutions which change their resistance. (4) In this method not the volume of red cells is considered in estimating the percentage of hemolysis, but their hemoglobin content, since the color of solutions with a partial hemolysis is compared with the color of a completely hemolyzed solution of the same volume of the same blood. This makes the method independent of any changes in the hemoglobin content of red cell which may change for instance by the change of their water content. (5) The red cells are not separated from blood; every separation from blood requires either a shaking with beads which decreases the resistance of red cells (l. c.) or a citration which submit the cells to abnormal conditions and change their resistance to hypotony. (6) As the red cells are not separated from blood, not only the changes of fragility produced by some changes in their protoplasm but those produced by some changes in the media surrounding them, are considered in the method presented.

In two experiments taken from the author's not yet published paper it was shown that outer conditions (sun light, high temperature) change the fragility of red cells very distinctly. Red cells are generally considered as real cells the protoplasm of which has the same physicochemical properties as that of colorless cells. It is therefore very probable that the fragility of colorless cells of our organism is changed similarly.

In the fragility test commonly used in clinical work, only the threshold of hemolysis is determined. Since there can be no doubt that the threshold value is not representative of the total or average fragility of red cells, it might be expected that the clinical use of the method presented here, will possibly change some of the current concepts on pathologic alterations of erythrocytes and it may show that alterations occur under certain physiologic or pathologic conditions in which they have not been known to occur.

REFERENCES

1. Hamburger, H. J.: Bestimmung der Resistenz der roten Blutkörperchen. Abderhalden's Handbuch der biologischen Arbeitsmethoden, Lif. 106, p. 263.
2. Lepeschkin, W. W.: Die Ursachen der Haemolyse. Medd. fr. K. Vet. Akad. Nobelinstit, Stockholm, **6**: Noll, 1, 1924.

AN IMPROVED METHOD FOR THE DETERMINATION OF BLOOD UREA NITROGEN BY DIRECT NESSLERIZATION*

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INTRODUCTION

THE almost universal recognition of the fact by clinicians that the diminution of blood urea clearance gives evidence of diminishing kidney function earlier than either the phenolsulphonphthalein excretion test[1] or the Mosenthal specific gravity fixation test,[2] leaves the modern hospital laboratory swamped with a gradually increasing number of requests for urine urea and blood urea nitrogen determinations. Of the blood constituents determined today, none has greater importance than urea nitrogen as one of the factors necessary in determining blood urea clearance as a clinical measure of kidney dysfunction. Of the hosts of methods proposed in recent years, the fact remains that the determination of blood urea nitrogen is still one of the most troublesome procedures encountered in the hospital laboratory. A contributing factor to the lack of uniformity in blood chemical methods is the failure of workers to realize that certain methods are better adaptable than others to multiple determinations, as may be required in clinical laboratories in office and hospital, while other more complicated methods giving a higher degree of accuracy may be better adaptable to scientific research. In so far as the clinical application of the data obtained is concerned, "undetermined nitrogen" and amino acid nitrogen, most remarkable for its constancy in all of the pathologic conditions thus far investigated, would hardly justify distilling off the ammonia from urease-treated tungstic acid blood filtrates before nesslerization in blood urea nitrogen determinations.

From the viewpoint of speed in emergency cases, ease and practicability of manipulation, direct nesslerization would be the method of choice for determining urea nitrogen in blood filtrates if the formation of turbid solutions could be prevented. Several such methods have been proposed, among them the method of Karr,[3] and its modifications by Roe and Irish,[4] Looney,[5] and others, the original method of Karr and its various modifications making use of the tungstic acid blood filtrate of Folin and Wu[6] in almost all cases. As has been pointed out before by Roe and Irish, Looney, and others, the difficulty encountered in Karr's method is the formation of turbid solutions after the addition of Nessler's reagent, making accurate colorimetric comparison with the nitrogen standard impossible in most cases. To overcome this difficulty many clarifying procedures have been proposed.

Proceeding on the assumption that the turbidity obtained on the direct nesslerization of urease-treated tungstic acid blood filtrates was due to the "precipitation of mercury in Nessler's reagent by traces of proteins, and other organic

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matter, in both the urease solution and the substrate,'' Roe and Irish attempt to solve the turbidity problem by calcium phosphate adsorption of the turbidity producing substances. An extra step is introduced in centrifugation and decantation of the material before nesslerization. In our hands, and in the hands of other workers (Looney, et. al.), this method is only partly successful in preventing turbidity.

Approaching the turbidity problem in Karr's method from another angle, Looney saw new possibilities in Folin's[7] use of gum ghatti as a protective colloid to prevent precipitation of Prussian blue in his (Folin's) micro sugar method, and employs this gum as a stabilizing colloid to prevent precipitaton of the complex ammonium mercuric iodide compound in Nessler's reagent by the turbidity producng substances present in urease-treated tungstic acid blood filtrates. In Looney's method the use of an additional substance (gum ghatti) as a protective colloid in blood filtrates adds to complications because the standard nitrogen solution must contain the same concentration of this substance as the blood filtrate, otherwise the decrease in intensity of color on nesslerization is quite apparent, about 10 per cent as pointed out by Looney. Furthermore, the same concentration of the alcoholic solution of urease must be added to the nitrogen standard as to the blood filtrate to guarantee an equal decrease in intensity of color due to this substance on nesslerization. Instead of the aeration method as used in routine work giving figures that are too low, as pointed out by Looney, we believe the contrary is true: that Looney's modification gives figures that are too high due to a greater decrease in color intensity on nesslerization of the nitrogen standard than on nesslerization of the blood filtrate, caused by the same concentration of gum ghatti and alcoholic urease solution. One could hardly guarantee an equal decrease in color intensity on nesslerization by adding the same substances, even in equal amounts, to two so widely differing solutions as a simple nitrogen standard and tungstic acid blood filtrates of varying complexity.

The failure of direct nesslerization methods in urease-treated tungstic acid blood filtrates point to the conclusion that before ammonia produced from urea by urease can be nesslerized it must first be separated from interfering urease proteins. We believe that we have been successful in accomplishing this separation by the use of whole oxalated blood and a specially purified and highly concentrated urease reagent, which is completely removed with the total proteins of the blood, according to the Folin-Wu technic of precipitation with tungstic acid (formed by the interaction of sodium tungstate and sulphuric acid) and filtration.

We were especially encouraged to undertake such studies by the researches of Sumner,[8-11] whose work suggested the application of a specially purified and highly concentrated urease reagent as a possible solution of the turbidity problem.

PREPARATION OF THE SPECIAL UREASE REAGENT

Shake 75 gm. ''Arlco'' (defatted) jack bean meal with 400 c.c. of a 32 per cent acetone solution at 28° C. for five minutes. Filter through a fluted filter paper in the refrigerator. Filtration is rapid and the filtrate should come through clear. Add 15 c.c. of N/10 acetic acid to the acetone filtrate and allow to remain on ice overnight. Centrifuge and pour off mother liquor. Wash pre-

cipitated urease twice by stirring up with ice cold 32 per cent acetone-phosphate solution, made by diluting c. p. acetone to 32 per cent with a phosphate mixture containing 143 c.c. of 0.1 M NaOH and 500 c.c. of 0.2 M KH_2PO_4, and centrifuging. (In place of the acetone-phosphate solution, one may wash sediment with ice cold 32 per cent acetone containing 5 per cent N/10 acetic acid, with a reasonably good yield). As pointed out by Sumner, the iso-electric point of urease is somewhere in the neighborhod of P_H 6.1, and the precipitated urease is least soluble in the acetone-phosphate solution mentioned above. Pour off the acetone phosphate mixture and drain tubes against filter paper until free from the odor of acetone. Tubes may be placed in the incubator at 37.5° C. for a few minutes to free from acetone, if care is exercised not to let the precipitated urease dry, which rapidly inactivates it. Unlike the crude urease preparations, it cannot be dried on paper without complete inactivation. Suspend the washed precipitate of urease in 5 c.c. of ammonia-free distilled water. Add 0.1 c.c. of toluene containing 5 per cent thymol as a preservative and keep tightly stoppered on ice in a small bottle. The preparation will keep on ice for at least a month, and in our hands such preparations have developed only a negligible amount of ammonia. The washed sediment will contain the urease in a highly concentrated form (globulin?), and should not give a test for ammonia nitrogen or free carbohydrate.

One drop of the concentrated urease preparation is used for each 5 c.c. of whole oxalated blood to be analyzed, and represents a urea hydrolyzing power of 178 times the 30 per cent alcoholic 2.5 per cent jack bean extract used in the method of Folin and Svedberg,[12] when made from the same jack bean meal and dropped from pipettes delivering 22 drops per cubic centimeter. One drop of the preparation produced 75.3 mg. of urea nitrogen from 25 c.c. of a 3 per cent urea solution, buffered with 5.4 per cent of K_2HPO_4 and 4.25 per cent of KH_2PO_4 in fifteen minutes at 50° C.

DETERMINATION OF BLOOD UREA NITROGEN

Transfer one volume (5 to 10 c.c.) of oxalated blood to a flask which has been rinsed in nitric acid and distilled water to free it from mercury. Add one drop of the special urease reagent to each 5 c.c. volume of blood taken for analysis, and two volumes of ammonia-free distilled water. Stopper tightly and incubate in a water-bath for fifteen minutes at 50° C. Remove from water-bath and add five volumes of water. Add one volume of 10 per cent sodium tungstate solution and mix. Add, with constant shaking, one volume of two thirds normal sulphuric acid. Stopper flask and allow to stand for five minutes. According to the Folin and Wu method of preparing a protein-free blood filtrate, if the color of the coagulum does not gradually change from bright red to dark brown, 10 per cent sulphuric acid must be added from a pipette one drop at a time, shaking after each drop, and continuing until there is practically no foaming and until the dark brown color is apparent. The amount of 10 per cent sulphuric acid that one finds it necessary to add, provided too much oxalate was not used as an anticoagulant, depends on the amount of urea present in the blood which has been hydrolyzed to ammonium carbonate by the urease, and rarely exceeds 4 or 5 drops even in blood high in urea nitrogen.

Pour the mixture on a dry filter paper. The filtrate should be perfectly clear and represents blood diluted 1:10. Place 5 c.c. of the filtrate in a Pyrex tube graduated at 25 c.c., or a 25 c.c. volumetric flask, add 15 c.c. of water, and nesslerize with 2.5 c.c. of Nessler's solution. Dilute to a volume of 25 c.c. No trace of turbidity should occur on nesslerization. The nitrogen standard for comparison is prepared at the same time by placing 4 c.c. of standard ammonium sulphate solution* (containing 0.4 mg. N) in 100 c.c. volumetric flask. Dilute to about 75 c.c., add 10 c.c. of Nessler's solution, and dilute to the mark. Compare in a colorimeter.

Calculation:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 0.4 \times \frac{100}{0.5} \times \frac{25}{100} = \text{mg. urea N per 100 c.c. blood.}$$

When 5 c.c. of the blood filtrate is used, the formula, on solving, becomes:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 20 = \text{mg. urea N per 100 c.c. blood.}$$

If the blood filtrate contains an unusual amount of urea nitrogen, of course it is necessary to repeat the nesslerization procedure, using less of the filtrate, in order to obtain a color comparable with the nitrogen standard.

The blood filtrate contains all of the constituents of the blood determined by the Folin-Wu system, without the objectionable presence of urease proteins or other interfering organic matter, and the same filtrate may be used in determining nonprotein nitrogen, uric acid, creatinine, glucose, and chlorides.

EXPERIMENTAL

Two typical experiments will now be cited to show that when the specially concentrated urease preparation is added to whole oxalated blood, it is completely

TABLE I

SHOWING THAT WITH THE IMPROVED METHOD OF DIRECT NESSLERIZATION OF FILTRATE FROM UREASE-TREATED WHOLE BLOOD ONE OBTAINS APPROXIMATELY THE SAME BLOOD UREA NITROGEN VALUES AS IN THE AERATION METHOD

Results Are Expressed as mg. Urea Nitrogen per 100 c.c. Blood

BLOOD NO.	UREASE USED DROPS	DIRECT METHOD	AERATION METHOD
1	1	10.4	10.2
2	1	11.0	11.0
3	1	13.0	12.9
4	1	30.2	29.6
5	1	23.0	24.0
6	1	11.8	11.4
7	1	43.7	45.1
8	1	100.0	98.2
9	1	125.5	122.9
10	1	156.3	152.8
11	1	123.0	122.0
12	1	134.3	132.6
13	1	152.0	149.3
14	1	202	198.8

In all determinations the concentrated urease suspension was dropped from a pipette delivering 22 drops per cubic centimeter.

*The standard nitrogen solution is prepared by dissolving 0.4716 gm. of purified ammonium sulphate in a liter of ammonia-free water, and is the same standard nitrogen solution commonly used in nonprotein nitrogen determinations.

removed by precipitation with tungstic acid along with the total proteins of the blood:

1. Thirty-five cubic centimeters of ammonia-free distilled water was added to 5 c.c. of the concentrated urease suspension, followed by 5 c.c. of 10 per cent sodium tungstate and 5 c.c. of two-thirds normal sulphuric acid. The precipitate which formed was filtered off and the total nitrogen present in the filtrate was determined by the micro-Kjeldahl method, employing direct nesslerization. Five cubic centimeters of the filtrate, after the micro-Kjeldahl procedure, gave no color on nesslerization.

2. Five cubic centimeters of the concentrated urease suspension and 5 c.c. of whole blood containing 25 mg. of nonprotein nitrogen per 100 c.c. of blood were mixed. Thirty cubic centimeters of ammonia-free distilled water was added, followed by 5 c.c. of 10 per cent sodium tungstate and 5 c.c. of two-thirds normal sulphuric acid. The precipitate which formed was filtered off and the nonprotein nitrogen again determined on the filtrate, which still remained 25 mg. per 100 c.c. of blood. It is worth while to recall here that only one drop of the enzyme preparation is used for each 5 c.c. of blood analyzed, which is sufficient to hydrolyze more than the amount of urea present in 5 c.c. of whole blood within upper pathologic limits.

TABLE II

SHOWING RECOVERY OF ADDED UREA BY THE IMPROVED METHOD OF DIRECT NESSLERIZATION

Results expressed as mg. urea nitrogen per 100 c.c. of blood

BLOOD UREA N.	UREA ADDED	TOTAL UREA PRESENT	UREA FOUND	UREA RECOVERED
mg. per 100 c.c.	mg. N	mg. N	mg. N	per cent
22.5	18.6	41.1	41.6	101.1
22.5	74.7	97.2	96.8	99.5
22.5	93.4	115.9	115.3	99.4
22.5	119.8	142.3	143.8	101

TABLE III

COMPARISON OF NONPROTEIN NITROGEN IN BLOOD WITH THE SAME BLOOD TO WHICH ONE DROP OF SPECIAL UREASE REAGENT WAS ADDED AND INCUBATED FOR FIFTEEN MINUTES AT 50° C.

Results expressed as mg. of nonprotein nitrogen per 100 c.c. of blood

SAMPLE NO.	FOLIN-WU FILTRATE	FOLIN-WU FILTRATE FROM UREASE TREATED WHOLE BLOOD
1	30	29.8
2	32	33
3	35	35
4	33	33
5	51	51
6	156	153

TABLE IV

SHOWING THAT THE GLUCOSE CONTENT OF WHOLE BLOOD IS NOT AFFECTED BY THE SPECIAL UREASE REAGENT ON INCUBATION FOR FIFTEEN MINUTES AT 50° C.

Results are Expressed as mg. of Glucose per 100 c.c. of Blood

SAMPLE NO.	FOLIN-WU FILTRATE	FOLIN-WU FILTRATE FROM UREASE TREATED BLOOD
1	74	74
2	68	68
3	194	194
4	191	188
5	195	195
6	98	96
7	100	97
8	84	85
9	99	102
10	300	297

TABLE V

SHOWING THAT THE SPECIAL UREASE REAGENT HAS NO EFFECT ON BLOOD GLUCOSE ON STANDING FOR TWELVE HOURS AT ROOM TEMPERATURE, OR ON INCUBATION FOR FIFTEEN MINUTES AT 50° C.

Results expressed as mg. glucose per 100 c.c. of blood

SAMPLE NO. (SAME BLOOD)	UREASE USED	ALLOWED TO STAND AT ROOM TEMPERATURE	INCUBATED AT 50° C.	GLUCOSE
5 c.c.	drops	hours	minutes	mg.
1	0	0	0	97
2	0	0	15	96
3	1	0	15	96
4	0	12	0	82
5	1	12	0	83

TABLE VI

SHOWING THAT RESULTS FOR URIC ACID AND CREATININE ARE NOT INFLUENCED BY THE CONCENTRATED UREASE REAGENT ON STANDING FOR TWELVE HOURS AT ROOM TEMPERATURE, OR ON INCUBATION FOR FIFTEEN MINUTES AT 50° C.

Results expressed as mg. per 100 c.c. of blood

SAMPLE NO. (SAME BLOOD)	UREASE USED	ROOM TEMPERATURE	INCUBATED AT 50° C.	URIC ACID	CREATININE
5 c.c.	drops	hours	minutes	mg.	mg.
1	0	0	0	3.4	1.5
2	0	12	0	3.4	1.6
3	0	0	15	3.4	1.5
4	1	12	0	3.3	1.6
5	1	0	15	3.4	1.5

That such a blood filtrate is sufficiently free of urease proteins, and other interfering organic matter, to justify its use in any of the determinations formerly made on the tungstic acid blood filtrate from blood to which no urease was previously added is indicated by Tables III to VI.

TABLE VII

SHOWING THAT THE TOTAL BUFFERING EFFECT OF WHOLE BLOOD IS SUFFICIENT TO ALLOW THE CONCENTRATED UREASE TO COMPLETELY HYDROLYZE THE AMOUNT OF UREA PRESENT WITHIN HIGH PATHOLOGIC LIMITS, WHEN INCUBATED FOR FIFTEEN MINUTES AT 50° C., AND THAT THE ADDITION OF ANY SPECIAL BUFFER SOLUTION IS UNNECESSARY

Results in mg. urea nitrogen per 100 c.c. of blood

BLOOD UREA N	UREA ADDED	TOTAL UREA PRESENT	UREA FOUND	UREASE USED	PHOSPHATE BUFFER USED*
mg. per 100 c.c.	mg. N	mg. N	mg. N		
17.1	186.9	204	206.0	1 drop	none
17.1	186.9	204	202.2	2 drops	none
17.1	186.9	204	200.1	4 drops	none
17.1	186.9	204	201.6	0.5 c.c.	none
17.1	186.9	204	200.8	0.5 c.c.	0.5 c.c.
17.1	186.9	204	204.2	0.5 c.c.	1.0 c.c.
17.1	186.9	204	197.9	0.5 c.c.	1.5 c.c.
17.1	186.9	204	205.3	0.5 c.c.	2.0 c.c.

*The phosphate buffer used consisted of 6.9 gm. of $NaH_2PO_4 \cdot H_2O$ and 17.9 gm. of $Na_2HPO_4 \cdot 12H_2O$ dissolved in 100 c.c. of ammonia-free water. All tests were made on 5 c.c. samples of the same blood.

SUMMARY

1. A new method for the determination of urea nitrogen in blood has been developed. The urea in whole oxalated blood is hydrolyzed to ammonium carbonate by the action of a specially purified and highly concentrated urease reagent in fifteen minutes at 50° C. The concentrated urease (globulin?) is completely removed with the total proteins of the blood by precipitation with tungstic acid, in the preparation of the Folin-Wu protein-free blood filtrate. Urea nitrogen is determined colorimetrically, without turbidity interference, after nesslerization of the blood filtrate.

2. The use of a protective colloid to prevent turbidity due to precipitation of the complex ammonium mercuric iodide in Nessler's reagent by urease proteins in blood filtrates is obviated.

3. The method requires no more blood than is required by other methods, and compares favorably in speed and accuracy with any existing method.

4. The method offers a degree of ease and practicability of manipulation which allows many determinations to be made at the same time, requires no additional apparatus, and eliminates technical difficulties and inaccuracies of distillation procedures.

5. The total buffering effect of whole oxalated blood is sufficient to maintain

a reaction suitable to allow one drop of the concentrated urease reagent to completely hydrolyze the amount of urea present in 5 c.c. of blood, even within upper pathologic limits, without the addition of any special buffer solution to the blood.

6. The blood filtrate contains all of the other constituents of the blood determined by the Folin-Wu system, unchanged, and the same blood filtrate may be used in such determinations.

7. The purified and highly concentrated reagent requires a minimum amount of time for preparation, particularily in consideration of its keeping qualities on ice, when preserved with 0.1 c.c. of toluene containing 5 per cent thymol for each 5 c.c. of the reagent.

REFERENCES

1. Rowntree and Geraghty: J. Pharmacol. & Exper. Therap. **1**: 231, 1910.
2. Mosenthal, H. O.: Arch. Int. Med. **16**: 733, 1915; Ohio State Med. J. **18**: 348, 1922.
3. Karr, W. G.: J. Lab. & Clin. Med. **9**: 3, 1924.
4. Roe, J. H., and Irish, O. J.: J. Lab. & Clin. Med. **11**: 1087, 1926.
5. Looney, J. M.: J. Biol. Chem. **88**: 189, 1930.
6. Folin, O., and Wu, H.: J. Biol. Chem. **38**: 81, 1919.
7. Folin, O.: J. Biol. Chem. **77**: 421, 1928.
8. Sumner, J. B., Graham, V. A., and Noback, C. V.: Proc. Soc. Exper. Biol. & Med. **21**: 551, 1924.
9. Sumner, J. B.: J. Biol. Chem. **69**: 435, 1926.
10. Sumner, J. B.: J. Biol. Chem. **70**: 97, 1926.
11. Sumner, J. B.: J. Biol. Chem. **76**: 149, 1928.
12. Folin, O., and Svedberg, A.: J. Biol. Chem. **88**: 77, 1930.

SUPRAVITAL DIFFERENTIAL COUNTING ADAPTED TO CLINICAL USE*

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THE supravital method for differential counting has not been generally adopted for clinical use because of two drawbacks in the technic usually employed. The first of these is that the differential must be made shortly after preparing the blood film, since it is a common experience that after the lapse of about two hours the cells have taken up large amounts of the dye and cannot be identified. The second is the requirement of a specially constructed warm chamber which is expensive and often is not available. A method has been developed which eliminates these difficulties. This method is presented here, together with comparative results obtained by its use.

METHOD

The method proposed differs from that usually employed[1, 2] in three respects: First, in the use of a more dilute solution of the dye; second, the preservation of the blood film in the ice box until the count can be made; and third, the substitution of a simple lighting system for the warm chamber. The stock solution of dye is made up in the usual way, 125 mg. of neutral red iodide No. 2 dissolved in

*From the Laboratories of The Rockefeller Institute for Medical Research.

50 c.c. of absolute alcohol. The slides are coated with a dye consisting of 30 drops of the stock solution in 10 c.c. of absolute alcohol instead of the usual 100 drops.* When the blood film has been prepared and sealed with vaseline as by the ordinary method, the count may be made at once, or the preparation may be stored in the ice box at a temperature of about 4° C. until ready for the examination. In making the counts a warm chamber is unnecessary. As the source of illumination, an ordinary 40 watt frosted electric light bulb suspended on an iron stand has been found inexpensive and satisfactory.

Through the use of this procedure, a preparation which has been kept in the ice box for twenty-four hours appears to be entirely similar to a fresh smear and cannot be distinguished from it; all cells have been found to be actively motile and to retain their morphologic, viable characteristics. Smears left in the open laboratory at ordinary room temperature (20° C.) were found to last about four hours or twice as long as those kept at 37° in the warm chamber. Poor results in delayed counts by this method are usually due either to improperly cleaned slides and cover-slips or to thick smears.

COMPARATIVE RESULTS

The accuracy of delayed differential counts is evidenced by our experience summarized in Tables I and II. In a preliminary report[3] parallel observations on six male rabbits were recorded. A series of 16 smears was counted immediately after taking the blood, with the usual technic, and after twenty-four hours in the ice box, a duplicate set of smears was counted with the modifications here described. In making this comparison, 5300 cells were counted on a total of 32 smears. The means and standard errors of the means of the two series of counts are given in Table I.

TABLE I

RESULTS OF IMMEDIATE AND DELAYED SUPRAVITAL COUNTS. FIRST SERIES

	NEUTROPHILES		BASOPHILES		EOSINOPHILES		LYMPHOCYTES		MONOCYTES	
Age of Smear in Hours	0-2	18-24	0-2	18-24	0-2	18-24	0-2	18-24	0-2	18-24
Means	59.0	57.6	3.6	4.7	0.9	0.8	23.4	24.7	12.5	12.0
Standard Error of the Mean	±2.1	±1.4	±0.4	±0.6	±0.2	±0.2	±2.2	±1.9	±1.1	±1.4

That comparable results were obtained is seen from the fact that no significant difference between the respective means was obtained, and in no case was this difference equal to twice its standard error.

Further experience with this method has confirmed our original observations. Duplicate sets of smears were made on 14 normal male rabbits, 3 rabbits infected with Treponema pallidum, 3 normal men, and 3 men with active syphilis, 46 smears in all. A total of 3400 cells was counted immediately on one set of

*The following is the method of preparing the slides and blood films as described by Sabin: The surface of a clean slide is flooded with the diluted stock dye solution. The excess of dye is drained off the slide which is then allowed to dry. Slides stained in this manner keep indefinitely. Blood films are made by placing a small drop of blood on a clean cover-slip which is then inverted gently on the slide. The cover-slip is rimmed with vaseline and the blood films are now ready for study.

smears, following the usual technic, and 3400 cells were counted on the duplicate set after preservation in the ice box for twenty-four hours. The results are shown in Table II.

TABLE II

RESULTS OF IMMEDIATE AND DELAYED SUPRAVITAL COUNTS. SECOND SERIES

	NEUTROPHILES		BASOPHILES		EOSINOPHILES		LYMPHOCYTES		MONOCYTES	
Age of Smear in Hours	0-2	18-24	0-2	18-24	0-2	18-24	0-2	18-24	0-2	18-24
Means	53.2	54.1	4.1	4.6	2.3	2.2	30.4	29.6	9.8	9.3
Standard Error of the Mean	±1.9	±1.6	±0.6	±0.8	±0.6	±0.5	±1.9	±1.6	±0.8	±0.9

Here again, there is no significant difference between the respective means, and this difference in no case equals twice its standard error.

The method has also been employed in isolated examinations of blood from sources other than those shown in the tables. Excellent results have attended delayed differentials made on rabbits inoculated with a malignant tumor, on rabbits with severe snuffles, on men with various anemias, myelogenous leucemia, infectious mononucleosis, and syphilis.

SUMMARY

A modification of the supravital technic for differential counting is here proposed which makes available to the clinician and general practitioner a method heretofore largely limited to scientific laboratories.

It renders possible accurate differential white blood cell counts with the supravital technic as long as twenty-four hours after making the preparation. This is accomplished (1) by reducing the concentration of the dye used, (2) by placing the blood smears in the refrigerator until ready for counting, and (3) by substituting a simple lighting arrangement for the warm chamber.

REFERENCES

1. Simpson, M. E.: Vital Staining of Human Blood With Especial Reference to the Separation of the Monocytes, Univ. of Calif. Pub. in Anat. **1**: 1, 1921.
2. Sabin, F. R.: Studies of Living Human Blood Cells, Bull. Johns Hopkins Hosp. **34**: 277, 1923.
3. Casey, A. E., and Rosahn, P. D.: Delayed Differential Counting of the White Blood Cells by a Modified Supravital Technique, Proc. Soc. Exper. Biol. & Med. **28**: 658, 1931.

NEW HARTMAN TRANSFUSION APPARATUS*

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A NEW apparatus for citrate transfusion has been evolved which embodies the advantages of citration at the needle described in previous publications[1, 2] and increases the simplicity, smoothness, and safety of the procedure.

DESCRIPTION OF APPARATUS

A one liter Pyrex bottle (*B*) with a connection at the bottom for the blood tube (*A*) is used. This blood tube consists of a No. 22 catheter. There is a needle connection at the end as illustrated. This connection and that with the bottle are tied to withstand the pressure used in giving the blood. The top assembly (*D*) is the regular aspirator bottle type. It has the usual two way connection with the bottle and valves which open and close the same. Small additional handles are placed on these valves to facilitate smooth operation. Attached to the core of the top assembly (*D*) a glass dropper and tube (*C*) are attached with a heavy rubber connection covering the end of the core but leaving the side opening through which suction or pressure may be produced in the bottle by attaching a two way aspirating pump to tube and connection (*F*). On the lower end of the dropper and tube (*C*) a small No. 8 catheter is attached which is cut long enough to run through the blood tube (*A*) to the connection at the end. Now it is seen that, with the connection and tube (*E*) which is long enough to reach the bottom of the citrate flask (*H*), a complete citrate system is formed so that when negative pressure is produced in the bottle, citrate is drawn from flask (*H*) through (*E*) and (*C*) to the end of the small catheter where it is released in the blood tube (*A*) and in turn reaches the bottle (*B*). If blood is coming from the vein through (*A*) at this time the citrate is mixed with the blood as it leaves the needle and flows to bottle (*B*).

The top assembly is held in place by a clamp (*K*) and screwed into position by a set screw (*G*). The end of this set screw (*G*) is attached to the top assembly (*D*), so that during sterilization the top assembly may be held suspended, thus avoiding molding of the rubber stopper. The citrate flask (*H*) is held by a spring-steel clamp which may be detached. Attached to this clamp is a scale showing the volume of fluid in the bottle. The citrate flask is graduated and holds 120 c.c.

OPERATION OF APPARATUS FOR TRANSFUSION

The apparatus is carefully washed and rinsed with distilled water. Then, with the bottle (*K*) half full of water, the small catheter is allowed to flow into the blood tube with the water. The entire apparatus, except the pump and needles, is wrapped and steam sterilized at 15 pounds for twenty minutes. Care must be taken not to bend tubes sharply or sterilize at higher temperatures. At

*From Department of Laboratories, Henry Ford Hospital.
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the time of use the top assembly (*D*) is screwed down into place with (*G*). The citrate flask (*H*) is filled with 2 per cent citrate solution. A two way aspirating pump is attached to (*F*) and negative pressure within the bottle, sufficient to draw citrate through the apparatus and leaving 15 c.c. in the bottle (*B*), is produced. If necessary the gloved finger or a sterile sponge may be held over the end of (*A*) during this procedure. The arm of the donor is now prepared and a blood pressure cuff placed above the elbow. The cuff is distended above diastolic

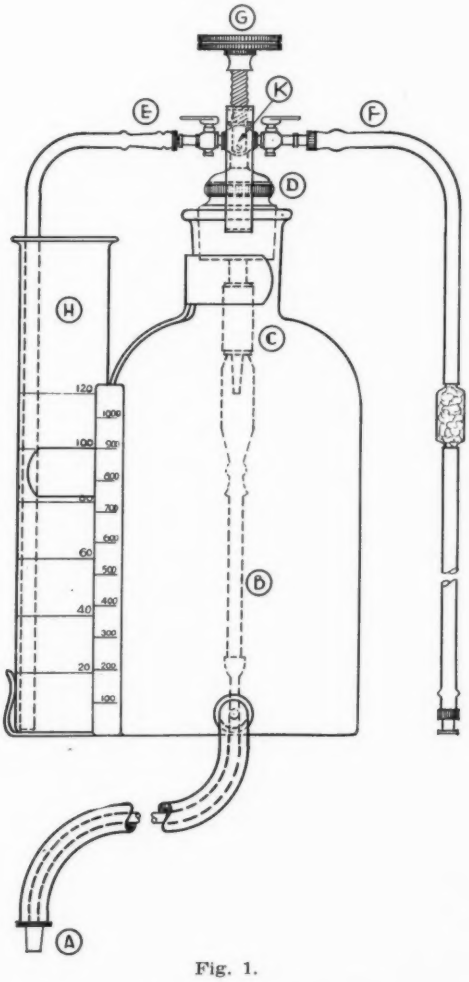

Fig. 1.

pressure and the needle plunged cleanly into the best available vein. When a good flow of blood is obtained tube (*A*) is attached to the needle. More negative pressure is now produced within the bottle (*B*) to obtain a good flow of blood and also to bring the citrate over from (*H*) through (*C*). The flow of citrate is observed as it comes through the dropper and must be kept flowing constantly, but the amount may be regulated by the valve shown near (*E*), so that there will be approximately 12 to 15 c.c. of the 2 per cent citrate to each 85 c.c. of blood drawn. The bottle may be gently rotated but never shaken.

When the desired amount of blood is obtained the pump is disconnected and the negative pressure released. The pressure in the blood pressure cuff is released and the blood tube (*A*) compressed between the fingers and the needle withdrawn from the donor's vein. The tube (*A*) is held high for drainage, and placed in clip in side of top assembly clamp (*K*) to prevent the loss of blood from the bottle (*B*).

The patient's arm is prepared and a needle placed in the vein. The blood tube (*A*) is lowered until it is filled with blood, then the connection is made with the needle. The aspirating pump is reversed, and, after closing the citrate system with the valve near (*E*), positive pressure is produced in the bottle (*B*) sufficient to effect a steady flow of blood into the patient's vein.

SUMMARY

A new apparatus for citrate transfusion is presented which has the advantages of citration at the needle thus preventing precoagulative changes but which avoids the use of the special double shouldered needle thus allowing the selection of any needle according to the vein of the donor. The dropper is placed within the bottle and the top assembly is such that there is no difficulty in holding the stopper in place even when considerable pressure is exerted within the bottle. The citrate is regulated by the small valve near (*E*) instead of the screw clamp. (This apparatus has been modeled for me by Mr. Cox of J. F. Hartz Company, 1529 Broadway, Detroit, Michigan, and may be obtained from them.)

REFERENCES

1. Hartman, F. W.: New Methods for Blood Transfusion and Serum Therapy, J. A. M. A. **71**: 1658-1659, 1918.
2. Hartman, F. W.: Transfusion Reactions and Citration Within the Needle, J. A. M. A. **78**: 15-18, 1922.

A SIMPLE VACUUM-TUBE POTENTIOMETER FOR THE MEASUREMENT OF GLASS ELECTRODE POTENTIALS*

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IN THE measurement of the potentials of the glass electrode it is necessary to employ a method which not only is sensitive but also draws no appreciable current from this high-resistance electrode. The advantages of the method given in this paper lie in the simplicity of the circuit and the ease of operation. These advantages depend upon the employment of the General Electric Company's "Pliotron" FP 54. It was later discovered that Hill[1] used this same tube for a similar purpose. However, the circuit which we employ is somewhat simpler; moreover, the only shield required, enclosing the tube, control-grid switch, and glass-electrode lead to the control-grid switch, forms a single compact unit.

The control-grid of this vacuum tube is very highly insulated and is stated by the makers to have a leakage-resistance of about 10^{16} ohms. This character-

*From the Pharmacological Laboratory and the Department of Physics, University of Chicago, Chicago.
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istic, of course, is of the greatest importance and enables one to make measurements at the "free" grid potential without further complication of the circuit since any negative potential in this tube is essentially a "free" grid potential. The current drawn by the control-grid is of the order of 10^{-15} amperes. Besides the filament, the tube also contains a space-charge grid. The filament, space-charge grid, and plate can all be operated from a single 6-volt storage battery since there is a potential of only 6 volts on the plate. The mutual conductance of the tube is 25 microamperes per volt.

A diagram of the circuit is shown in Fig. 1 in which a Leeds and Northrup type K potentiometer is employed. The control-grid switch (SW_1) is described in detail below. Switch 2 (SW_2) enables one to reverse the current of the potentiometer cell. Switch 3(SW_3) is required when the galvanometer is used for the standardization of the potentiometer current. B_1 is a lead storage battery. B_2 is a 1.5 volt dry cell (a Burgess "C" battery is satisfactory). B_3, B_4,

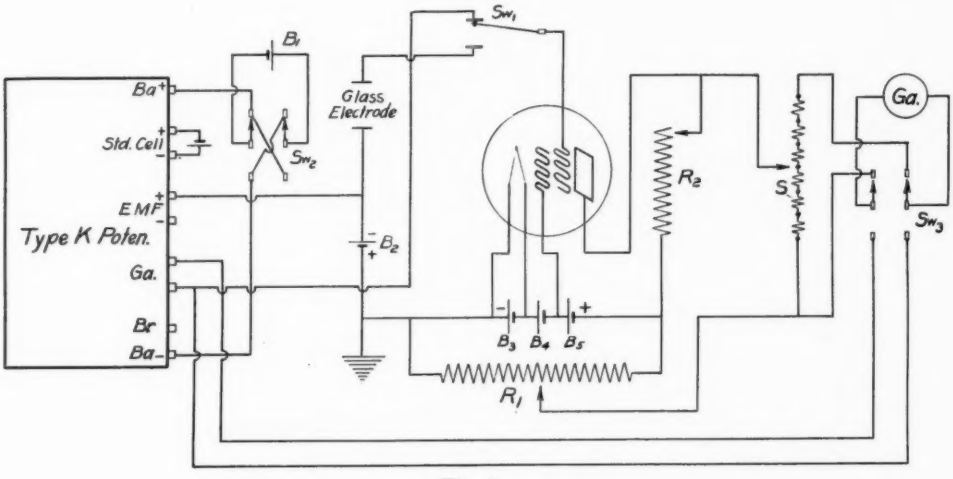

Fig. 1.

and B_5 are the three cells of an automobile storage battery. The potential divider R_1 should have a total resistance of several thousand ohms to avoid undue drain on the battery. It should be wire-wound. R_2 is a resistance of about 10,000 ohms which may be kept fixed. S is an Ayrton shunt of a resistance (3,000 ohms) appropriate for the galvanometer used. The galvanometer is a Leeds and Northrup type R instrument with a sensitivity of 0.003 microampere per millimeter.

The tube and control grid switch (SW_1) must be completely shielded. A satisfactory arrangement is shown in Fig. 2. All dimensions are given in inches. The parts labelled A, B, and C, are made of brass and are grounded through a binding post soldered in A. After a careful cleansing with alcohol and ether, the tube is kept dry by means of P_2O_5. A phosphor-bronze strip is soldered to the control-grid lead at the top of the tube. Into the free end of this strip is soldered a platinum pin which makes contact with E and F through platinum. Contact through F is made by depressing the quartz-rod plunger D. The binding post of F may be used for connecting it with the silver wire of the silver-silver chloride

half-cell of a glass electrode of the type devised by MacInnes and Dole[2] if the electrode is inserted in the electrode-thermoregulator of Stadie, O'Brien and Laug.[3] On the other hand, if one wishes the glass electrode to be held in a vertical position, a side-arm consisting of two pieces of brass tubing soldered together at a right angle and containing a copper wire imbedded in sulphur may be slipped over C. One end of the copper wire is fastened in the binding post of F; the other end (in the vertical limb of the tube) is clipped to the silver wire of a glass electrode which is immersed in the solution, the P_H of which is being determined. Wires are soldered to the four prongs in the base of the tube for connecting the filament terminals, the space-charge grid, and the plate to the storage

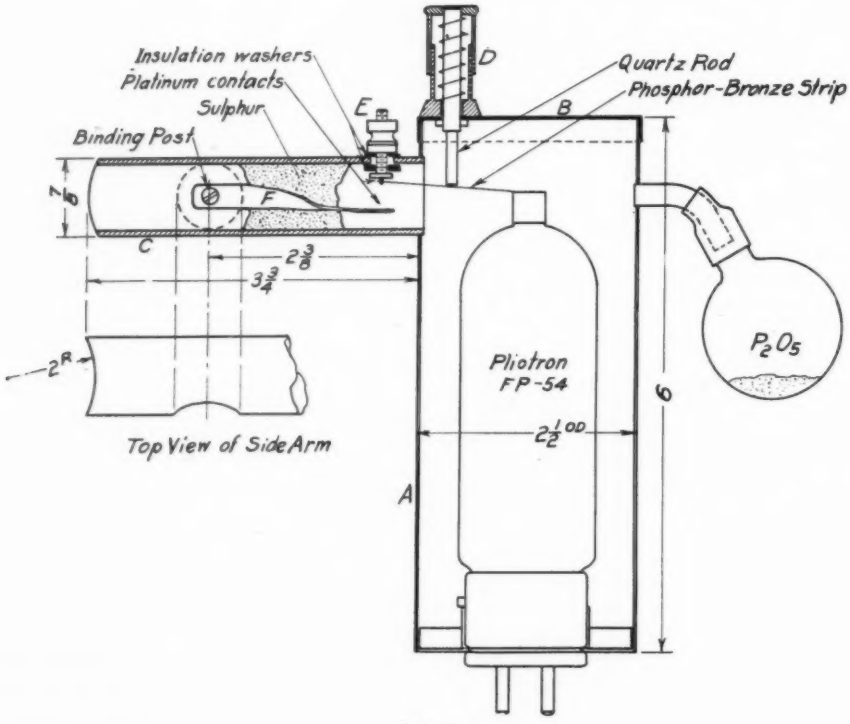

Fig. 2.

battery; these connections may remain permanently and need not be interrupted while the battery is being charged.

OPERATION OF THE POTENTIOMETER

The principle of the method employed is to apply alternately to the control-grid a known fraction of the potential of the potentiometer-cell B_1, and the unknown potential of the glass electrode. When these two potentials are identical the grid potential will remain constant when the plunger is depressed. Consequently, the plate current of the tube will also remain constant. The object of the branch circuit R_2, R_1, S, and Ga is to determine when such constancy exists.

The action of the circuit is briefly as follows: the plate current through R_2 produces a potential drop across it of a few tenths of a volt, so that the actual

plate-potential is a few tenths less than the total of batteries B_3, B_4, B_5. The slider on R_1 is then adjusted to give this same potential. If now the galvanometer is connected between the slider and the plate, it will show no deflection. Further if SW_1 is now depressed and the choice of potentiometer voltage was correctly made so that the potentials of E and F (Fig. 2) are identical, the galvanometer still will not deflect. Under these conditions the potential of the glass electrode, being the same as that of E (Fig. 2), can be read directly on the potentiometer. The purpose of the Ayrton shunt is to reduce the sensitivity of the galvanometer during preliminary adjustments.

The adjustment need not be made so that the galvanometer deflection is actually zero with the Ayrton shunt set for maximum sensitivity. It must, however, be sufficiently near zero to keep the image on the scale. The important requirement is that the image does not move as SW_1 is depressed.

TABLE I

COMPARISON OF DETERMINATION OF P_H OF PHOSPHATE BUFFERS BY HYDROGEN AND GLASS ELECTRODES

NO.	P_H BY HYDROGEN ELECTRODE*	P_H BY GLASS ELECTRODE
1	6.441	6.430
2	7.067	7.055
3	7.275	7.270
4	7.478	7.466
5	7.664	7.659
6	7.860	7.841

*Determined by Dr. M. E. Hanke.

Under conditions of maximum sensitivity for the shunt a change of potential of 1 millivolt causes a deflection of the galvanometer image of 4.4 to 4.7 mm. If the potential of the control-grid bias (B_2) is increased to −3 volts, the sensitivity is reduced to 2.9 to 3.1 mm. per millivolt. This is because at the greater grid bias the tube operates on a less steep part of the grid potential-plate current characteristic. The drift of the galvanometer image is not sufficient to interfere with estimates of the accuracy indicated in the examples given below. While the maximum drift which has been observed is 0.3 mm. per minute, ordinarily it is about 0.2 mm. per minute.

In Table I are given examples of determinations of the P_H of buffer solutions by the hydrogen electrode and by the glass electrode. The glass electrode determinations were made at room temperature and undoubtedly could be improved by more adequate temperature control. Some idea of the reproducibility of determinations is furnished by eight successive determinations on a phosphate-buffer of P_H 7.51; the P_H values found were 7.505, 7.508, 7.513, 7.512, 7.502, 7.497, 7.497, and 7.509. These also were determined at room temperature.

REFERENCES

1. Hill, S. E.: Use of an Improved Null Instrument for the Glass Electrode or Other High Resistance Circuits, Science **73**: 529, 1931.
2. MacInnes, D. A., and Dole, Malcolm: The Behavior of Glass Electrodes of Different Compositions, J. Am. Chem. Soc. **52**: 29, 1930.
3. Stadie, W. G., O'Brien, H., and Laug, E. P.: Determination of P_H of Serum at 38° With the Glass Electrode and an Improved Electron Tube Potentiometer, J. Biol. Chem. **91**: 243, 1931.

A CLASS EXPERIMENT ON CO-ENZYMIC ACTIVITY*

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THE armamentarium of reliable laboratory experiments which can be used by senior students in the study of enzymic processes to demonstrate for themselves the specific effect of an activator on an enzymic reaction, is somewhat limited.

The following experiment, which takes advantage of the very striking effect of Mg ions on the phosphatase activity of rats' (or dogs') red blood cells[1] has been recently devised for such students and may be carried out without difficulty in any laboratory possessing a colorimeter.

MATERIAL REQUIRED

(a) Six cubic centimeters of rats' or dogs' red cells (beef or human red cells give a much smaller activation) from oxalated or defibrinated blood. These are washed once with 0.85 per cent NaCl solution, and laked by adding 18 c.c. of distilled water containing chloroform, and if necessary by freezing and thawing. After keeping for one hour the laked or almost completely laked preparation is centrifuged, and the upper layer removed from the stromata, calcium oxalate, and unlaked cells.

(b) One hundred fifty cubic centimeters of a 0.30 per cent solution of sodium β-glycerophosphate (or the commercial glycerophosphate will serve), adjusted to P_H 7.4.

(c) Eighty-five one hundredths per cent sodium chloride solution.

(d) A few cubic centimeters of M/5 (approx.) magnesium chloride solution, and the same quantity of M/5 (approx.) calcium chloride solution, both adjusted to P_H 7.4.

(e) Twenty-five per cent trichloracetic acid solution.

METHOD

In Table I are given directions for setting up the experiments. Ordinary test tubes fitted with rubber stoppers are used.

Since in the absence of added enzyme no hydrolysis of sodium glycerophosphate takes place in twenty-four hours under these conditions, controls containing glycerophosphate (with and without $MgCl_2$ or $CaCl_2$) but no red cell extract have been omitted from Table I, but may be set up at the same time if thought desirable.

PROCEDURE

To four tubes A, A_1, E, and E_1, 2 c.c. of 25 per cent trichloracetic acid are added immediately. After mixing and standing for not more than fifteen minutes, the contents of these four tubes are filtered and the filtrates, carefully stoppered, are kept in the refrigerator. Filtration without undue delay is necessary to avoid errors which may arise from the hydrolytic action of the trichloracetic acid, even in the cold, on the lipins of the red cells.[2] All the rest of the tubes are

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TABLE I

DIRECTIONS FOR SETTING UP A CLASS EXPERIMENT TO DEMONSTRATE MAGNESIUM ACTIVATION OF PHOSPHATASE

NO. OF TUBES	TUBE LABELS	0.3% NA β-GLYCERO-PHOSPHATE	WATER	0.9 % NaCl	M/5 $MgCl_2$	M/5 $CaCl_2$	DILUTED RED CELLS	$CHCl_3$ DROPS	TYPICAL RESULTS Mg. P	SHOWING
2	A, A_1	10	–	2	–	–	1	3	0.022	
2	B, B_1	10	–	2	–	–	1	3	0.074	Hydrolysis in absence of Mg.
2	C, C_1	10	–	1.8	0.2	–	1	3	*0.304*	Marked activation by approx. 0.003 M Mg.
2	D, D_1	10	–	1.8	–	0.2	1	3	0.063	Slight inhibition by approx. 0.003 M Ca.
2	E, E_1	–	10	2	–	–	1	3	0.021	Autolysis controls
2	F, F_1	–	10	2	–	–	1	3	0.053	
2	G, G_1	–	10	1.8	0.2	–	1	3	0.056	
2	H, H_1	–	10	1.8	–	0.2	1	3	0.055	

incubated in a water thermostat at 38° C. for from twelve to twenty-four hours, with occasional mixing. At the end of this time the tubes are cooled to room temperature, 2 c.c. of trichloracetic acid are added to each tube, and the contents mixed. After standing fifteen minutes the contents of these tubes are filtered through phosphate-free filter papers, and the inorganic phosphate is determined on aliquots (10 c.c. or less) of the filtrates and of the filtrates from A, A_1, E, and E_1, using any convenient micromethod for inorganic P determination (e. g. Briggs, Bell-Doisy, Fiske-Subbarow). It is advisable to separate the aliquots into duplicate series of eight filtrates each, and to use 0.1 and 0.3 mg. P as standards for 10 c.c. aliquots. For convenience in reading, 0.05 mg. P is added to all the flasks containing aliquots except C before adding the colorimetric reagents, and is then subtracted after the reading has been made.

Typical inorganic P contents of 10 c.c. aliquots are shown in Table I. In this particular experiment rats' red cells were used. An activation of several hundreds per cent is shown.

FURTHER EXPERIMENTS

Since most of the autolyzable phosphoric esters of the red cells are hydrolyzed under the experimental conditions just described, it is not shown by this experiment whether or not the presence of $MgCl_2$ activates the autolysis ("Phosphatolysis") process. This point can be taken up with the senior students if desired. The effect of other cations related to Mg may be examined also, and it will be found that the activation effect is, apparently, quite specific for Mg.

REFERENCES

1. Jenner, H. D., and Kay, H. D.: The Phosphatases of Mammalian Tissues III. Magnesium and the Phosphatase System, J. Biol. Chem. **93**: 733, 1931.
2. Kay, H. D.: A Source of Error in Nitrogen and Phosphorus Determinations on Filtrates Obtained After Precipitation of Tissue Colloids by Trichloracetic Acid or Other Strong Acid, J. Biol. Chem. **93**: 727, 1931.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

HEPATIC FUNCTION: Van den Bergh Reaction and Bromsulphalein Test in the Estimation of, Cantarow, A. Am. J. M. Sc. **184**: 215, 1932.

Cantarow compares these two methods in the study of hepatic function in 188 patients and concludes that the analysis of observations on the degree of bilirubinemia, bromsulphalein retention and the van den Bergh reaction in a group of 188 patients showing abnormal response to one or more of these studies appears to indicate that the type of diazo reaction is not determined solely by the serum bilirubin content.

The type of van den Bergh reaction appears to bear a more direct relation to the degree of bromsulphalein retention than to the degree of bilirubinemia except in cases of extra-hepatic obstructive jaundice.

There is no direct correlation between the type of van den Bergh reaction and the plasma cholesterol concentration.

Repeated determinations of the van den Bergh reaction and the observation of its transition from one type to another in individuals with biliary tract disease are of considerable significance, if interpreted in conjunction with the estimation of the degree of bilirubinemia and bromsulphalein retention.

Of 234 patients with cholecystitis with and without cholelithiasis, 70 presented either hyperbilirubinemia or bromsulphalein retention or both. Of these, 34 were cases of chronic cholecystitis, without cholelithiasis and with no other demonstrable evidence of hepatic disease.

In 3 cases of gall-bladder disease there was 100 per cent retention of bromsulphalein at the end of 30 minutes, associated with icterus index values of 8.3, 7.2 and 8.7. These findings suggest dissociation of these two phases of the excretory function of the liver.

This disturbance of hepatic function in patients with gall-bladder disease appears to be largely "functional" in nature and associated with but slight demonstrable organic disease of the liver parenchyma.

More careful routine preoperative study of patients with surgical disorders of the biliary passages and proper preoperative treatment will perhaps greatly diminish the postoperative morbidity and mortality of biliary tract surgery.

LIPEMIA: Variations in the Total Blood Lipid. Wechsler, H. F. Arch. Int. Med. **50**: 37, 1932.

The total blood lipid curves after the ingestion of 100 c.c. of olive oil in sixty-seven subjects who were apparently free from pancreatic or hepatic disease could be subdivided into three categories: ascending, 63.2 per cent; flat, 17.5 per cent, and descending, 19.3 per cent.

The age of the subject and the presence of arteriosclerosis markedly influenced the total blood lipid. Persons in the second and third decades presented a flat curve, those in the fourth, fifth and sixth decades an ascending curve and those showing evidence of arteriosclerosis a flat or descending curve.

Starvation produced a moderate progressive rise in the total blood lipid.

Dextrose eliminated the starvation effect.

CULTURE MEDIUM: From Soy Bean, Kostriko, D. S., and Maryash, T. K. Lab. Pratika, Moscow **8**: 10, 1932.

The following is described as an inexpensive medium efficacious for the growth of most pathogenic bacteria, especially suitable for anaerobes, and for bacterial counts:

Three methods of preparation are described:

1. A soy bean extract is prepared by adding 100 gm. of soy beans to 1 liter of water, boiling from thirty minutes to one hour, filtering, and sterilizing by autoclaving twenty minutes at 120° C.

2. Several soy beans (eight or ten) are added to a test tube with water and sterilized like the extract.

3. Soy bean agar is prepared by adding from 1.5 to 2 per cent agar to the extract and sterilizing.

BLOOD SEDIMENTATION TEST in the Management of the Pneumothorax Patient, Cutler, J. W. Am. Rev. Tub. **26**: 134, 1932.

The sedimentation test was studied in a group of 131 patients receiving artificial-pneumothorax treatment during the past three and a half years. The purpose in view was to ascertain what help, if any, the physician may expect from the test in the management of such cases. The following observations and deductions were made:

1. The sedimentation test fills a gap in the management of tuberculous patients receiving artificial-pneumothorax treatment. It gives valuable information at a time when x-ray findings are obscured and physical signs and symptoms obliterated by the collapse.

2. The sedimentation rate is a sensitive measure of the activity of the tuberculous process in the compressed lung and is the last objective evidence of activity to become normal. A return to normal indicates quiescence of the pathologic process but does not necessarily indicate stability of the lesion.

3. In uneventful cases the sedimentation rate shows steady improvement and changes from a vertical or a diagonal curve, indicating activity of the tuberculous process, to a horizontal line, indicating quiescence, but not until weeks or months after the disappearance of constitutional signs and symptoms.

4. When there is disease in both lungs and the more-involved lung has responded to pneumothorax treatment, the sedimentation rate is a simple means to determine the effect of the compression on the less involved side. It will not become normal until the tuberculous process is quiescent in both lungs.

5. During the early part of the treatment the sedimentation test should be repeated at least once a month and afterward every two months. A diagonal line with an index of 15 mm. or more should always be respected. The patient should be warned that the disease is active and that a relapse is possible. Exercise should be prescribed with great caution and its effect studied carefully by means of the sedimentation test regardless of how well the patient may feel clinically. Adherence to this rule will prevent many a relapse and spread in the opposite lung.

6. The test is simple to perform and to interpret. By its proper utilization the physician can gather valuable information out of all proportion to the effort made to obtain it. Further study should make this one of the most widely used clinical laboratory procedures in phthisiotherapy.

TUBERCLE BACILLI: Rapid Staining of, in Sputum, Doglio, P. Giorn. di Batt. e Immun. Turin, **8**: 243, 1932.

Applying the principle, first proposed by Spengler, that red acid-fast organisms are readily detected against a yellow background, Doglio proposes the following contrast stain:

Brilliant yellow	0.15 gram
Sulphuric acid conc.	10 c.c.
Alcohol	20 c.c.
Distilled water	85 c.c.

This is used after carbol fuchsin in the usual manner, as both a decolorizing solution and control stain. The bacilli are red on a lemon-yellow background.

GONORRHEA: Unreliability of Laboratory Aids in the Diagnosis of, in Women, Jacoby, A. Am. J. Obst. & Gynec. **23**: 729, 1932.

The following conclusions are advanced:

1. Repeated smears should be taken and carefully examined.

2. The use of Gram's stain is not essential. In conjunction with a proper evaluation of the clinical examination, the methylene blue stain is adequate for practical purposes.

3. A positive smear is conclusive evidence of infection.

4. A negative smear, even when repeated, does not exclude the presence of a gonococcal infection in women.

5. Suspicious organisms, extra- or intracellular, should be interpreted in accordance with the clinical evidence.

6. Pure spreads of pus cells, even without organisms present, should be regarded as suspicious evidence of gonococcal infection.

7. When cultures are taken and prove positive, they constitute criteria, but are not practical or well adapted to routine practice.

8. A negative culture does not exclude the presence of a gonococcal infection.

9. The complement fixation test for gonorrhea with the present technic is unreliable. Neither positive nor negative findings are conclusive.

10. Unless an improved technic affording more reliable results is evolved, the fixation test should not be used for the diagnosis of gonorrhea or for the control of its treatment.

Even under the most favorable conditions, it is apparent that laboratory procedures are of minor importance in establishing a diagnosis of gonorrhea in women. A wider appreciation of this fact, with a consequently greater reliance upon the history and clinical evidence, will suggest the correct diagnosis in many of the now unrecognized cases of gonorrhea in women.

TUBERCLE BACILLI: A Simplified Egg Medium for the Cultivation of, Feldman, W. H. Am. Rev. Tub. **26**: 187, 1932.

PREPARATION OF MEDIUM

Seven fresh eggs are washed in warm water and immersed for ten minutes in 80 per cent alcohol.

A portion of the shell is carefully broken at one end of the eggs, and the membranous sac is punctured with sterile, sharp-pointed scissors. The egg-white is discarded and the yolks are discharged into a sterile mixing bowl.

To the egg-yolks are added 100 c.c. of a 6 per cent solution of glycerin prepared as follows: glycerin 24 c.c., distilled water 500 c.c., sterilized in the autoclave for fifteen minutes at 15 pounds' pressure. The solution can be autoclaved in 100 c.c. portions and stored for future use.

The egg-yolks and the solution of glycerin are thoroughly mixed with a sterile egg beater and tubed, using sterile apparatus. Precautions are taken to minimize possible contamination.

The sterilizing is done in the Arnold sterilizer or the inspissator, on the first day at 75° C. until solidified, then at 85° C. for one hour, and on the second, third and fourth days at 75° C. for one hour. Before the medium is used it should be incubated for two days at 37° C. It is best stored in the refrigerator, and drying can be minimized by placing the tubes in large cylindrical museum jars, smearing the contact surfaces of the jar and the lid with petrolatum. A few crystals of thymol seem to assist materially in controlling molds.

SEDIMENTATION TEST: A Comparison of the Sedimentation Test and Ruge Virulence Test in 150 Gynecologic Cases, Simunich, W. A. Am. J. Obst. & Gynec. **23**: 724, 1932.

In the Ruge virulence test 5 c.c. of blood drawn from the cubital vein under aseptic precautions is defibrinated by shaking for five minutes in a sterile glass tube containing glass beads. One-half c.c. of this blood is placed in each of two tubes one of which is inoculated with 2 or 3 loopfuls of vaginal or cervical secretion and the other is kept as a control. Smears are made from the contents of both tubes which are immediately thereafter incubated at 37.5° C. Smears are repeated at hourly intervals for three hours and stained with methylene blue. If the organisms increase within three hours the test is positive; i. e., organisms pathogenic for the host are present in the genital canal.

The following conclusions are drawn from a study of 150 cases:

1. An increase in sedimentation speed of 60 minutes or less was observed in more than 50 per cent of the cases of inflammatory adnexitis, uncomplicated and complicated fibroids and the carcinomata, and in about 23 per cent of other abdominal and vaginoabdominal pathology not of an inflammatory nature.

2. The presence of virulent organisms is one of the most important causes of postoperative morbidity and mortality but a doubtful or positive virulence test does not depend on the speed of sedimentation.

3. The increase in sedimentation speed is due to some other factor than the virulence of organisms.

4. The sedimentation test is not a reliable guide in the determination of the time for safe operation of adnexal disease.

5. The Ruge virulence test is of value in the prognostication of postoperative morbidity and mortality if the operation takes place at the site of the organisms, usually the cervix.

6. The history, white and differential counts, temperature and physical examination must remain our main guides in the determination of the time for safe operation in adnexal disease, while in cervical and combined cervicoabdominal operations the Ruge virulence test is of undoubted value. A doubtful or positive virulence test contraindicates cervical operations until such a time that the test becomes negative.

BLOOD: Blood Picture in Sickle Cell Anemia, Diggs, L. W. Southern M. J. **25:** 615, 1932.

The blood elements in sickle cell anemia, although subject to wide variations, present during the definitely anemic phases of the disease a diagnostic blood picture. Sickled cells in the fixed smear are rarely specific enough to make the diagnosis on morphology alone. The suspicion is usually aroused by the history and clinical signs, by an anemia associated with jaundice, leucocytosis and premature erythrocytes in the smear. Given such a combination in a negro, the demonstration of the sickle cell phenomenon in the moist preparation clinches the diagnosis. Reticulocyte count, cell volume, fragility test and examination of the family for sickling would be confirmatory procedures indicated. It is predicted that sickle cell anemia will become a common diagnosis when clinicians become sickle cell anemia minded, and in addition to personally studying blood smears, make moist preparations routine in unexplained hemolytic anemias.

The characteristic features of the blood picture in active sickle cell anemia are:

(a) The presence of sickled cells in moist preparations.

(b) Signs of red blood cell destruction (poikilocytosis, degenerating forms, microcytes, increased serum bilirubin, negative direct van den Bergh and positive indirect; urobilinuria, phagocytosis of erythrocytes by large mononuclears).

(c) Signs of increased regenerative activity on the part of the bone marrow (megaloblasts, intermediary forms, nucleated red blood cells, nuclear fragments of all types, diffuse basophilia macrocytes, increased reticulocytes, leucocytosis, with a shift right and left, increased platelets).

Sickling without anemia, hemolytic anemia without sickling, or secondary anemia in association with the sickle cell trait are to be distinguished from true sickle cell anemia.

Typical sickled cells, although striking and unmistakable in fixed smears and fresh moist preparations from some cases, are the exception rather than the rule.

The average red blood cell in sickle cell anemia is smaller than normal. The cell volume is decreased out of proportion to the decrease in the red blood cell count and hemoglobin. The color index is variable but usually below 1.

The erythrocytes in sickle cell anemia are more resistant to hypotonic salt solutions than normal.

SUGAR TOLERANCE, Phosphates In, McCullagh, D. R., and Van Alstine, L. Am. J. Clin. Path. **2:** 277, 1932.

The phosphate changes in the blood of normal individuals after the administration of glucose show considerable regularity.

The changes in blood phosphate after the administration of glucose to patients suffering from metabolic disorders frequently differ from the changes in blood phosphate in normal individuals.

It is impossible to make a definite differential diagnosis in various metabolic disorders by means of the phosphate curve.

B. ACIDOPHILUS, Medium for, Kulp, W. L., and White, V. Science 76: 17, 1932.

Mixture A.—Add 10 gm. Difco peptone and 10 gm. Difco peptonized milk to 400 c.c. of juice filtered from a good quality of canned tomatoes. Heat this mixture gently to dissolve the peptone and peptonized milk. Unnecessary heating of the tomato juice should be avoided. The reaction of the solution is changed to P_H 6.0 to 6.2. There should be little deviation from this suggested reaction.

Mixture B.—Add 11 gm. dried agar to 600 c.c. distilled water and autoclave this mixture to dissolve the agar.

Just previous to the removal of Mixture B from the autoclave, bring Mixture A to the boiling point. Then mix A and B while both are hot and filter through a thin layer of absorbent cotton. Distribute the filtered medium in containers (test tubes preferred) and sterilize by heating in the autoclave at 120° C. for eight minutes.

LEISHMANIA: A New Solid Medium for the Cultivation of L. donovani, Salle, A. J., Brit. J. Infect. Dis. 49: 473, 1932.

Peptone (Difco) 20 gm., beef infusion 250 c.c., salt 5 gm., agar 15 gm. and distilled water to make 830 c.c. are boiled to dissolve the agar. Water is added to replace loss by evaporation, and the reaction is adjusted to P_H 7.2. The agar base is distributed into flasks (83 c.c. to each flask) and sterilized in the autoclave. When required, to one flask is added 2 c.c. of sterile 50 per cent dextrose solution and the agar is melted. When cooled to 50° C. 15 c.c. of defibrinated rabbit blood is added and the mixture distributed in test tubes to solidify as slopes. On the surface of this medium in five or six days at 22° C. massive quantities of organisms are obtained. The liquid medium is composed of peptone 20 gm., beef infusion 250 c.c., salt 5 gm., dextrose 10 gm. and distilled water to make 550 c.c. The reaction is adjusted to P_H 7.2 with sodium hydroxide. To this mixture is added a mixture of 300 c.c. of distilled water and 150 c.c. of defibrinated rabbit's blood which has been allowed to stand till complete hemolysis has taken place. The resulting 1000 c.c. of medium is centrifuged till clear and sterilized by filtration through Seitz or Berkefeld filters. The medium should be used in a shallow layer at the bottom of a flask.

HEMOPHILIA: Mechanism of, in Infancy and Childhood, Kugelmass, I. N. Am. J. Dis. Child. 44: 50, 1932.

The following conclusions are presented:

1. The criteria necessary and sufficient for the diagnosis of hemophilia have been formulated on the basis of experimental and clinical studies.

2. Quantitative determinations of the constituents involved in blood coagulation show the hemophilic deficiency to be in the primary stage of the blood-clotting mechanism.

3. Hemophilic blood shows a strikingly low prothrombin content compensated by a correspondingly high antithrombin content. The platelets are normal in number but are physiologically defective having a slow rate of disintegration.

4. Hemophilic blood is characterized by a blood-clotting index that is less than one-tenth of the normal. The index constitutes the ratio of the concentration of the substances tending to clot over those tending to favor bleeding. Accordingly, the normal index of clotting function is 0.5, whereas the hemophilic clotting index is less than 0.05. This very low index of blood-clotting function is diagnostic of hemophilia.

5. The index of blood-clotting function shows a manifold increase after transfusion, but the improved clotting lasts for only forty-eight hours. Transfusion does not alter the clotting function of blood in hemophilic subjects with manifestations in the joints.

6. Dietary protein, lipids, vitamins or minerals do not alter the deficient clotting function in hemophilia.

7. Hemophilic persons show the absence of the female sex hormone in their tissues normally present in males. Ovarian therapy, theelin and other products of the female generative organs injected into the hemophilic subject produce no change in the clotting mechanism evaluated quantitatively.

8. Serum injected or applied locally is not effective in controlling hemorrhage in hemophilia, unless it is fresh and rich in thrombin. But nonhemophilic bleeders respond readily to any serum and to dietary protein therapy.

SUGAR TOLERANCE: Clinical Evaluation of Blood Phosphate and Sugar Tolerance Curves, Hartman, F. W., and Foster, D. P. Am. J. Clin. Path. 2: 289, 1932.

Five hundred combined glucose tolerance and phosphate curves were taken on patients considered potential diabetics. One hundred were from individuals 25 to 100 pounds overweight. These show an increased rise of the blood glucose with slow fall while the inorganic phosphates decreased moderately with slow recovery.

One hundred two combined glucose tolerance and phosphate curves from individuals, normal in weight or undernourished, showed high elevation of the glucose curve with gradual return to the fasting level while the phosphate curve showed only slight depression with slight recovery.

Twenty-five combined glucose tolerance and phosphate curves on mild or moderate diabetics showed typical diminished glucose tolerance curves. The phosphate curves showed slight and continued depression.

The curve of inorganic phosphates is a valuable supplement to the glucose tolerance curve in the diagnosis of abnormal carbohydrate metabolism.

LEUCOCYTES: White Blood Cell Counts in Convalescence From Infectious Diseases, Reznikoff, P. Am. J. M. Sc. 184: 167, 1932.

While admitting the usefulness and value of the hemograms emphasis is laid upon the fact that it is only one nonspecific factor and that its real value arises from sequential counts at frequent intervals rather than isolated determinations.

What a patient shows at a particular moment is only of importance insofar as it throws light on the direction a patient is going.

In recovery from an acute infectious disease three more or less distinct phases may be demarcated by the hemogram: subsidence of the acute stage, convalescence, complete return to normal.

The subsidence of the acute stage is characterized by a decrease in immature polymorphonuclears and a marked, even if transitory, rise in monocytes.

Convalescence is characterized by a normal immature polymorphonuclear count, a marked rise in lymphocytes (often above 40 per cent) and a variable eosinophilia.

Complete recovery is characterized by a return of the lymphocytes to a normal range.

MUSEUM SPECIMENS, Mounting of, Caylor, H. D. Science 75: 517, 1932.

Discarded x-ray film may be used for mounting of small specimens of light weight.

The emulsion is removed by soaking in hot water and scraping. The dried film is then cut to the desired size to make an exact fit for the inside of the museum jar, and the fixed specimen sewed in place. Fixing fluid is then added and the jar sealed. The advantages of the method are: (1) suspension of specimen on an invisible material; (2) film transparent, allowing view of both sides; (3) film is waste material; (4) the cumbersome glass frame is unnecessary.

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EDITORIAL

Ulcer Vs. Carcinoma

IT IS inevitable, and, from the nature of the situation inescapable, that in the consideration of medical matters in general there should be many instances in particular concerning which there is a difference of opinion.

A subject of perennial debate, of which much has been written and still more said, is that of gastric ulcer as concerns the proper, most advisable, and most satisfactory method of treatment. On this subject physicians and surgeons have carried on for years a perpetual debate of which the end is not yet in sight, for each has experience and statistics to support his contention, vigorously combated by the differing experience and statistical data of his opponent.

While it is relatively easy to outline generalities applicable to abstract concepts, it is not always easy to apply them successfully to particular instances, and so the discussion of the medical versus the surgical treatment of gastric ulcer continues with temporary victory resting now with one side and now with the other.

It cannot be disputed, of course, that there are cases in which the results of medical treatment, carefully planned and faithfully followed, have been eminently satisfactory from the clinical standpoint and in which no disagreeable aftermath has been recorded.

It is equally true that there are other cases which, despite the seemingly satisfactory results, eventually have reached the surgeon.

On the other hand, it is also true that the surgical treatment of ulcers has had its failures as evidenced by the recurring ulcers and the patients who have not found gastroenterostomy and its like a panacea.

It is not strange that this should be the case and that statistical data should show such wide and marked differences, for it is beyond question that many "cures" attributable to medical treatment, and an equal number following surgical intervention are "cures" only because no consistent effort is made to assure a thorough "follow-up" of the case, or because the patient, for one reason or another, passes out of observation, many later to appear in some other set of records.

In the last analysis gastric ulcer is not in itself a disease but the manifestation of an underlying mechanism, the precise nature of which remains to be demonstrated.

As always, it is not the disease which must be treated but the patient who has it, and as long as the conditions which lead to ulcer production continue to exist in a particular patient, no treatment can be considered as final in its results.

The surgeon who confronts the internist with the fact that his experience has failed to demonstrate complete epithelialization following medical treatment, may be confronted in turn by the case in which, despite a beautiful and efficient short-circuiting, recurring and severe hemorrhage dominate the later history.

The ulcer patients stand between the Scylla of hemorrhage on the one hand and the Charbydis of malignant transformation on the other and much of the complaint of the surgeon rests upon the fact that all too often the ulcer has become a carcinoma by the time it reaches his hands.

It is certainly true that there are many gastric ulcers which, for various reasons, economic or otherwise, may well be subjected to medical treatment. There are others, however, in which the beginning of malignant changes renders immediate operative interference imperative.

Obviously, then, the evolution of methods whereby the imminence of malignant transformation may be foretold would be of the highest practical significance.

The studies of Alvarez and MacCarty[1] on the relation of the size of gastric ulcers to the development of malignant changes are of great as well as practical interest.

This study was conducted to establish, if possible, the contention of MacCarty, Carman, and others of the Mayo Clinic that any chronic *gastric* ulcer with a crater more than 2.5 cm. in diameter (as established roentgenographically), is probably the seat of cancerous change and should be subjected to surgical rather than medical treatment.

It is quite apparent that, if this contention be true, a criterion is available

whereby malignancy arising from gastric ulcers may be properly treated while it is still in the operable stage.

From the percentage distribution curves based on areas of 638 resected gastric ulcers and 682 gastric carcinomas resected during this study, it appears that four out of five benign ulcers are less than 1.8 cm. in diameter (smaller than a dime), and ninety-two out of the one hundred are less than 2.4 cm. in diameter (smaller than a quarter).

Of the carcinomas resected in the series, 23 per cent fell within the range of size of benign ulcers so that, on the basis of size alone, there is one chance in ten that an ulcer smaller than a quarter is already cancerous; two to one that, if larger than a quarter but smaller than a silver dollar, it is a cancer; while it is almost certainly cancerous if larger than a dollar.

These studies indicate the essential importance of roentgenologic examination of all patients with persistent gastric symptomatology and surgical intervention in all who present ulcers with a crater more than 2.5 cm. in diameter, appreciating, of course, that even then cases will be missed because of inability to demonstrate the lesion with sufficient clearness to enable its accurate measurement.

REFERENCE

1. Alvarez, W. C., and MacCarty, W. C.: Size of Resected Gastric Ulcers and Gastric Carcinomas, J. A. M. A. **91**: 226, 1928.

—*R. A. K.*

ITEM

The American Society of Clinical Pathologists

The following men were made honorary members of the American Society of Clinical Pathologists:

Charles Achard, Professor of Clinical Medicine, at the Faculty of Medicine in Paris; member of the Institute and the Academy of Medicine.

Dr. F. Craig, Col. U. S. Army, Rtd., Director of Department of Tropical Medicine, Tulane University, New Orleans, La.

Dr. E. C. Dodds, M.V.C., M.D., Ph.D., B.S., B.Sc., M.R.C.P., Courtauld Professor of Biochemistry in the University of London, Chemical Pathologist to the Middlesex Hospital.